PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 August 1998 (06.08.98)	
International application No. PCT/IL98/00012	**Applicant's or agent's file reference** 108981
International filing date (day/month/year) 13 January 1998 (13.01.98)	**Priority date** (day/month/year) 14 January 1997 (14.01.97)
Applicant SAVION, Naphtali et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 July 1998 (19.07.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer N. Fischer
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

2172575

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel-Aviv
ISRAEL

RECEIVED
29 -04- 1999
REINHOLD COHN & PART

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Applicant's or agent's file reference 108981.2	Date of mailing *(day/month/year)* 20.04.99
	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL98/00012	13/01/1998	14/01/1997

Applicant
RAMOT UNIVERSITY AUTHORITY FOR APPLIED .. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Articl 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. (+31-70) 340-2040 Tx: 31 651 epo nl Fax: (+31-70) 340-3016	Kruydenberg, G Tel.(+31-70)-340-2277

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

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19

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
108981.2		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL98/00012	13/01/1998	14/01/1997
International Patent Classification (IPC) or national classification and IPC A61K38/17		
Applicant RAMOT UNIVERSITY AUTHORITY FOR APPLIED .. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 16 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/07/1998	20.04.99
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. (+31-70) 340-2040 Tx: 31 651 epo nl Fax: (+31-70) 340-3016	Authorized officer Fernandez y Branas,F Telephone No. (+31-70)-340

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL98/00012

I. Basis of the r p rt

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-3,7,8,10,11	as originally filed	
9,12-15	filed with the demand	
4-6	with telefax of	18/02/1999

Claims, No.:

1-67	with telefax of	18/02/1999

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 23-33, 35-37, 39-44.

because:

☒ the said international application, or the said claims Nos. 23-33, 35-37, 39-44 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	15, 18-22, 45-51, 53, 58, 66
	No:	Claims	1-11, 13-14, 17, 52, 54, 56-57, 60-62, 64-65
Inventive step (IS)	Yes:	Claims	15, 45-51, 58, 66
	No:	Claims	1-11, 13-14, 17-22, 52-54, 56-57, 60-62, 64-65
Industrial applicability (IA)	Yes:	Claims	1-11, 13-15, 17-22, 45-54, 56-58 (see Sep. Sheet), 60-62, 64-66
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

s e separate sheet

Re Item I
Basis of the report

The subject matter of claims 12, 34, 55, and 63 goes beyond the disclosure of the application as originally filed. The expression "reconstituted HDL comprising phospholipids and/or sphingolipids" has not been disclosed in the application as originally filed.
The subject matter of claims 16, 38, 59 and 67 also goes beyond the disclosure of the application as originally filed. The combination of the subject matter of said claims with the subject matter of the claims they depend of (12, 34, 55 or 63 respectively) give rise to matter which was not explicitly or implicitly present in the application as filed, see for example the combinations of human HDL and Apolipoprotein A-I or A-IV. The pharmaceutical compositions of claim 9 as originally filed, or the description at page 6 lines 1-8 do not support said subject matter.

Thus, the present preliminary examination report does not take into account claims 12, 16, 34, 38, 55, 59, 63 and 67 as their subject matter extends beyond the content of the application as originally filed in the sense of Rule 70.2(c).

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 23-33, 35-37, 39-44, relate to a method of treatment method of the human or animal body by therapy in the sense of Article 34(4)(a)(i) and Rule 67.1(iv) PCT.

Re Item V
Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1...OPTHALMOLOGICA 1990, 201:206-212
D2...ROTE LISTE 1995, Abstracts 51131 (Intralipid) and 51132 (Lipofundin)

D3...WO-A-9112808 (MACNAUGHT PTY. LIMITED)
D4...PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, VOL 90, 1993, 12.040-12.044

In the case of a known substance or composition, this may only be patented for use in methods of treatment of the human or animal body if the known substance or composition was not previously disclosed for use in therapy ("first medical use"). The same substance or composition cannot subsequently be patented for any other use of that kind.

Compositions such as Intralipid® and Lipofundin® have already been used in methods of treatment of the human or animal body, see D1, page 207, left column, third paragraph and D2, abstracts 51131 and 51132.

Phospholipids have also been used in compositions for artificial tears and treating "dry eye syndrome", see especially D3, page 3 line 3 to page 6 line 13 (treatment of sore eye, lubricant for post-cataract surgery and intra-ocular lenses). In D3 the compositions may contain as well hyaluronic acid (which may be considered an extracellular component).

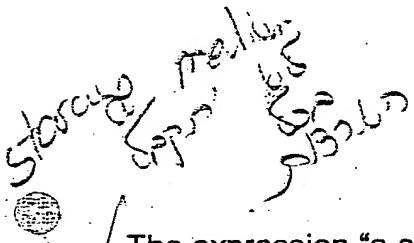
High Density Lipoprotein (HDL) and Apolipoprotein E have also been used in the said methods of treatment, see D4, front page abstract and page 12041, left column, "preparation of human Apo-HDL" and "reconstitution"

Thus, in view of D1, the subject matter of claims 8, 10 and 13 and lacks novelty in the sense of Article 33(2) PCT.

In view of D2, the subject matter of claims 8-10 and 13 lacks novelty in the sense of Article 33(2) PCT.

In view of D3, the subject matter of claims 8, 13-14 and 17 lacks novelty in the sense of Article 33(2) PCT.

In view of D4 the subject matter of claims 1-8, 11 and 13 lacks novelty in the sense of Article 33(2) PCT.



The expression "a storage medium for the preservation of isolated cornea" is understood as a medium "suitable for the storage and preservation of isolated cornea" see the Guidelines, C-III, 4.8. Bearing in mind this interpretation in view of D2 the subject matter of claims 60-61 and 64 lacks novelty in the sense of Article 33(2) PCT; In view of D3 the subject matter of claims 60-61 and 64-65 lacks novelty in the sense of Article 33(2) PCT; In view of D4, the subject matter of claims 60-62 lacks novelty in the sense of Article 33(2) PCT.

D1 discloses the use of compositions comprising Intralipid® as tear substitute materials. The use of phospholipids for the same purpose is also known from D3. Although none of these documents disclose the mechanism by which the Intralipid® or phospholipids act, (and certainly do not disclose the increase of cholesterol net efflux from cells, or the regeneration of the epithelial cells of the anterior segment of the eye), the said documents disclose the use of said compounds for preparing medicaments for treating disorders of the anterior segment of the eye, e.g. dry eye syndrome, lubricant for intra-ocular lenses, post cataract surgery, conjunctivitis, inflammatory conditions, (see D3, page 3, lines 3-15 and page 6 lines 7-13).

The Applicant is of the opinion that claim 52 as amended is restricted to indications which are completely different from D1 or D3. However, the said indications in claim 52 are vague and of unclear scope, see for example the expressions "mechanical abrasion of the cornea", or "corneal epithelial defects created by contact lens wearing"

or "(with) recurrent erosion of epithelium".

As the compositions of D1 or D3 are used as tear substitute materials and for the treatment of dry eye syndrome (D1 or D3), as lubricant for intra-ocular lenses (D3), in post cataract surgery (D3) and the treatment of conjunctivitis and inflammatory conditions (D3), they are meant to ameliorate or relieve conditions produced as a result of damage of the eye epithelium e.g. as a result of the abrasion produced by intra-ocular lens wearing, or in the dry eye as a result of the abrasion produced by the lack of lubrication. Thus, the indications of D1 or D3 fall within the vague definitions of claim 52.

Additionally, the discovery of a new mechanism of action does not prevent the fact that D1 or D3 disclose the same compositions for the same medical indication. The novelty of the claims cannot be restored under the disguise of another or newly specified pharmacological mechanism. In fact, the discovery of such new way of action does not in itself change the technical effect obtained.

Thus, in view of D1, the subject matter of claims 52, 54 and 56 lacks novelty in the sense of Article 33(2) PCT.

Thus, in view of D2, the subject matter of claims 52 and 56-57 lacks novelty in the sense of Article 33(2) PCT.

In view of the prior art the subject matter of claims 15, 18-22, 45-51, 53, 58 and 66 appears to be new in the sense of Article 33(2) PCT.

The subject matter of claims 21-22 and 53 is not considered to involve an inventive step in the sense of Article 33(3) PCT. Lipofundin® is considered an equivalent of Intralipid®, see in this sense D2; claims 21-22 do not add any matter that may be

considered to involve an inventive step over the disclosure of D1 or D3, mainly when the addition of other known compounds for protection e.g. from U.V. radiation would have been obvious for the skilled person.

It would appear also obvious for the skilled person to use the compositions disclosed in D1 or D3 in the disorders mentioned in claim 52 and not included in the novelty objection according to Article 33(2) mentioned above in the present report, as the skilled person would, apart from other treatments, use a tear substitute as protecting agent in many of these conditions, see in this sense D3, cited pages, supra.

For the analysis of the inventive step of claims 15, 45-51, 58 and 66 D1 is considered to be the closest prior art. The difference between the present application and D1 is that in the present application HDL are used in the preparation of pharmaceutical compositions for treating the conditions set forth in claim 47, or as storage medium when containing triglycerides and /or glycerol. In view of this difference the problem solved by the present application can be defined as the provision of alternative pharmaceutical compositions for treating eye disorders and the provision of alternative storage medium for the preservation of isolated corneas.

There is no suggestion in the prior art concerning the use of said HDL compounds or compositions in the treatment of the said diseases or in the preparation of a corneal storage medium and thus the subject matter of these claims involves an inventive step in the sense of Article 33(3) PCT.

The IPEA cannot appreciate at the present stage the distinguishing technical feature of claims 18-20 that could make the compositions of said claims inventive over the disclosures in D1 or D3, mainly when claims 18-20 are dependent of claim 8, and mainly when, as mentioned above in this report, the medical indications of

claim 8 (or claim 52) are vague and of unclear scope, see for example the expression "recurrent erosion of epithelium". Therefore the subject matter of claims 18-20 does not involve an inventive step in the sense of Article 33(3) PCT.

For the assessment of the presently worded claims 1-11, 13-15, 17-22, 45-54 and 56-58 on the question whether their subject matter is industrially applicable, no unified criteria exist in the PCT. The patentability under national patent laws can also be dependent on the formulation of the claims. The EPO, for example, does not recognise the subject matter of claims to the use of a compound in medical treatment as being industrially applicable, but will allow, however, claims to a known compound for first medical use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

The definition of the diseases in claims 1 and 45 as "disorders of the anterior segment of the eye" encompasses a high number of ill-defined malfunctions of the eye, as a result of which the scope of protection of said claims is not well defined, is interpretable and thus the said claims 1 and 45 are unclear in the sense of Article 6 PCT.

Paragraph ii) of claims 8, 52 and 60 is unclear. It is vague and thus unclear what is meant by "at least one other lipid component of HLD other than cholesterol and cholesterol-ester".

Lipofundin and Intralipid are trade marks. The said expressions do not convey any technical information for the skilled person and are therefore unclear, see Guidelines, C-III, 4.5b.

The expression "recurrent erosion of the epithelium" (claims 8, 52) is unclear.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel-Aviv
ISRAEL

RECEIVED
18-05-1998
REINHOLD COHN & PARTNERS

Applicant's or agent's file reference 108981	Date of mailing *(day/month/year)* 13. 05. 1998
International application No. PCT/IL 98/00012	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant RAMOT UNIVERSITY AUTHORITY FOR APPLIED .. et al.	International filing date *(day/month/year)* 13/01/1998

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Deborah Grandis

Form PCT/ISA/220 (January 1994)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
108981		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 98/00012	13/01/1998	14/01/1997

Applicant

RAMOT UNIVERSITY AUTHORITY FOR APPLIED .. et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Ir. .ational application No.
PCT/IL 98/00012

B x I Obs rvati ns wh re ertain claims w r found uns archabl (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 24-46
are directed to a method of treatment of
the human/animal body , the search has been carried out and based on the
alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 98/00012

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K31/66 A01N1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RIEGER G. : "Lipid-containing eye drops: a step closer to natural tears" OPTHALMOLOGICA, vol. 201, 1990, pages 201-212, XP002062710 see the whole document ---	1-9,11, 12,14, 22,23, 32,34, 37,55, 57,59, 63-65,68
X	BUNDESVERBAND DER PHARMAZEUTISCHEN INDUSTRIE E.V.: "ROTE LISTE 1995" 1995 , ECV.EDITIO CANTOR. AULENDORF/WÜRTT XP002062712 See abstracts 51131 and 51132 --- -/--	1-11,14, 16, 63-65, 68-70

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
21 April 1998	13.05.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/IL 98/00012

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 12808 A (MACNAUGHT PTY LTD) 5 September 1991 see the whole document	1-9,14, 15,32, 37,55, 59,60, 63,64, 68,69
X	EP 0 312 814 A (BOSTON OCULAR RES) 26 April 1989 see the whole document	1-9,14, 15,32, 37,55, 59,60, 63,64, 68,69
X	WO 94 04178 A (BIO TECHNOLOGY GENERAL CORP) 3 March 1994 see the whole document	1-9,17, 64,71
X	LEVINE D.M. ET AL: "In vivo protection against endotoxin by plasma high density lipoprotein" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, 1993, WASHINGTON US, pages 12040-12044, XP002062711 see the whole document	1-9,13, 14,16, 64,67,71
X	EP 0 733 918 A (BOSTON OCULAR RES) 25 September 1996 see the whole document	63,64
A	WO 95 14488 A (OCUTECH INC ;HAGEMAN GREGORY S (US)) 1 June 1995	1-71
A	EP 0 240 031 A (ALLERGAN INC) 7 October 1987 see the whole document	1-71

2

INTERNATIONAL SEARCH REPORT

....ormation on patent family members

Interm ...al Application No
PCT/IL 98/00012

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9112808 A	05-09-91	AU 655919 B	19-01-95
		AU 7307691 A	18-09-91
		EP 0516685 A	09-12-92
EP 0312814 A	26-04-89	US 4914088 A	03-04-90
		DE 3874479 A	15-10-92
		ES 2052664 T	16-07-94
		JP 1146824 A	08-06-89
		US 5578586 A	26-11-96
		US 5278151 A	11-01-94
WO 9404178 A	03-03-94	AU 673543 B	14-11-96
		AU 5007293 A	15-03-94
		CA 2141598 A	03-03-94
		EP 0659085 A	28-06-95
		JP 8502730 T	26-03-96
		NO 950491 A	30-03-95
		NZ 255732 A	26-05-97
		ZA 9305879 A	11-03-94
EP 0733918 A	25-09-96	JP 9101488 A	15-04-97
WO 9514488 A	01-06-95	AU 1294195 A	13-06-95
EP 0240031 A	07-10-87	CA 1295238 A	04-02-92
		DE 3787188 D	07-10-93
		DE 3787188 T	16-12-93
		ES 2044856 T	16-01-94
		JP 2661909 B	08-10-97
		JP 63005745 A	11-01-88
		US 4981841 A	01-01-91
		US 4983580 A	08-01-91

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel-Aviv
ISRAEL

RECEIVED
-4 -11- 1998
REINHOLD COHN & PARTNERS

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	28.10.98
Applicant's or agent's file reference: 108981.2	**REPLY DUE** within 3 months/~~days~~ from the above date of mailing
International application No.: PCT/IL 98/00012	International filing date *(day/month/year)*: 13/01/1998
Priority date *(day/month/year)*: 14/01/1997	
International Patent Classification (IPC) or both national classification and IPC: A61K38/17	
Applicant: RAMOT UNIVERSITY AUTHORITY FOR APPLIED .. et al.	

1. This written opinion is the First (first, etc.) drawn up by this International Preliminary Examining Authority.
2. This opinion contains indications relating to the following items:
   - I [X] Basis of the opinion
   - II [ ] Priority
   - III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [X] Certain defects in the international application
   - VIII [X] Certain observations on the international application
3. The applicant is hereby **invited to reply** to this opinion.

   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

   Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.
4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 14/05/1999

Name and mailing address of the IPEA/
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Netherlands
Tel.: (+31-70) 340-2040, Tx. 31 651 epo nl
Fax: (+31-70) 340-3016

Authorized officer / Examiner: J. Fernandez y Branas, F.
01948

Formalities officer (incl. extension of time limits): C. Cardenas
Telephone No.

I. Basis of the opinion

1. This opinion has been drawn up on th basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☐ the international application as originally filed

☒ the description, pages 1-3, 7-8, 10-11 ,as originally filed
pages 4-6, 9, 12-15 ,filed with the demand
pages , filed with the letter of

☒ the claims Nos. ,as originally filed
Nos. 1-54 ,as amended under Article 19
Nos. , filed with the demand
Nos. , filed with the letter of

☒ the drawings, sheets / fig. 1/9-9/9 ,as originally filed
sheets / fig. , filed with the demand
sheets / fig. , filed with the letter of

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.

☐ the drawings, sheets / fig.

3. ☒ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2 (c)).

1) The term "clinical damage" (claim 1, 19, 37). This term has not been disclosed in the application as originally filed and is vague enough to comprise an undefined number of alternatives not all present in the original disclosure.
2) The expression "and/or sphingolipids" within the framework of item ii) of claims 6, 24, 42 and 49. In the application as originally filed it is nowhere mentioned that the so called "reconstituted HLD" contains phospholipids and/or sphingolipids.

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 19-36

because:

☒ the said international application, or the said claims relate to the following Nos. 19-36 subject matter which does not require an international preliminary examination *(specify)*:

Claims 19-36 relate to a method of treatment of the human or animal body by therapy in the sense of Article 34(4)(a)(i) and Rule 67.1(iv) PCT.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims are so unclear that no meaningful opinion could be formed *(specify)*: Nos.

☐ the claims, or said claims are so inadequately supported by the description that no meaningful opinion could be formed. Nos.

☐ no international search report has been established for said claims Nos.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Claims	1-13, 37-41, 44, 48-54
	Claims	
Inventive Step	Claims	1-18, 37-41, 43-44, 48-54
	Claims	
Industrial Applicability	Claims	1-23, 37-47 (see below)
	Claims	

2. Citations and Explanations

D1...OPTHALMOLOGICA 1990, 201:206-212
D2...ROTE LISTE 1995, Abstracts 51131 (Intralipid) and 51132 (Lipofundin)
D3...WO-A-9112808 (MACNAUGHT PTY. LIMITED)
D4...EP312814 (OCULAR RESEARCH CORPORATION)
D5...PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, VOL 90, 1993, 12.040-12.044
D6...WO-A-9404178 (BIOTECHNOLOGY GENERAL CORP.)

Only applies to European practice

In the case of a known substance or composition, this may only be patented for use in methods of treatment of the human or animal body if the known substance or composition was not previously disclosed for use in therapy ("first medical use"). The same substance or composition cannot subsequently be patented for any other use of that kind.

Compositions such as Intralipid® and Lipofundin® have already been used in methods of treatment of the human or animal body, see D1, page 207, left column, third paragraph and D2, abstracts 51131 and 51132.

Phospholipids have also been used in compositions for artificial tears and treating "dry eye syndrome", (D3 and D4), see especially D3, page 3 line 3 to page 6 line 13 (treatment of sore eye, lubricant for post-cataract surgery and intra-ocular lenses). In D3 the compositions may

contain as well hyaluronic acid (which may be considered an extracellular component).

High Density Lipoprotein (HDL) and Apolipoprotein E have also been used in the said methods of treatment, see D5 and D6.

Thus, in view of D1, the subject matter of claims 1-5 and 8 lacks novelty in the sense of Article 33(2) PCT.

In view of D2, the subject matter of claims 1-5 and 7-8 lacks novelty in the sense of Article 33(2) PCT.

In view of any of D3 or D4, the subject matter of claims 1-5 lacks novelty in the sense of Article 33(2) PCT. In view of D3, the subject matter of claim 13 lacks novelty in the sense of Article 33(2) PCT.

In view of D5 the subject matter of claims 1-6 and 9-12 lacks novelty in the sense of Article 33(2) PCT.

In view of D6 the subject matter of claims 1- and 9-12 lacks novelty in the sense of Article 33(2) PCT.

The expression "a storage medium for the preservation of isolated cornea" is understood as a medium "suitable for the storage and preservation of isolated cornea" see the Guidelines, C-III, 4.8. Bearing in mind this interpretation, any document describing any of the compounds or compositions of claims 48-54 may be cited against the novelty of these claims, e.g. in view of D2 the subject matter of claims 48 and 50 lacks novelty in the sense of Article 33(2) PCT; The subject matter of claims 49 and 51-54 lacks novelty in view of D5 or D6.

Already rejected

D1 discloses the use of compositions comprising Intralipid® as tear substitute materials. The use of phospholipids for the same purpose is also known from D3 or D4. Although none of these documents disclose the mechanism by which the said compounds or compositions act, and certainly do not disclose the increase of cholesterol net efflux from cells, the said documents disclose the use of said compounds for preparing medicaments for treating

disorders of the anterior segment of the eye, e.g. dry eye syndrome, lubricant for intra-ocular lenses, post cataract surgery, conjunctivitis, inflammatory conditions, (see D3, page 3, lines 3-15 and page 6 lines 7-13). As the compositions of D1 and D3-D4 are used as tear substitute materials, they are meant to ameliorate or relieve conditions produced as a result of damage of the eye epithelium. The discovery of a new mechanism of action does not prevent the fact that D1, D3 or D4 disclose the same compositions for the same medical indication. The novelty of the claims cannot be restored under the disguise of another or newly specified pharmacological mechanism. In fact, the discovery of such new way of action does not in itself change the technical effect obtained.

Thus, in view of any of D1 or D3 or D4, the subject matter of claims 37-41, 44 lacks novelty in the sense of Article 33(2) PCT.

The subject matter of claims 17-18 and 43 is not considered to involve an inventive step in view of any D1 or D3 in the sense of Article 33(3) PCT. Lipofundin® is considered an equivalent of Intralipid®, see in this sense D2; claims 17-18 do not add any matter that may be considered to involve an inventive step over the disclosure of D1 or D3, mainly when the addition of other known compounds for protection e.g. from U.V. radiation would have been obvious for the skilled person.

The IPEA remarks that the present application provides no evidence that a direct relationship between the so called "cholesterol efflux" from cells and the therapeutic effect of the claimed compounds or compositions exists. Thus, claims 1-5, 13-18 and 37-41 are not considered to be supported by the description in the sense of Article 6 PCT; it is, for the same reason, not certain that all compounds or compositions capable of increasing a cholesterol efflux from cells will produce the desired technical effect (curing disorders of the anterior segment of the eye). Hence, it would appear that a part of the subject matter of claims 1-5, 13-18 and 37-41 does not solve any technical problem and therefore does not comply with the requirements of Article 33(3) PCT.

It would appear also obvious for the skilled person to use the compositions disclosed in D1 or D2 or D3 in some of the disorders mentioned in claim 3 and 39 of the present application, as the skilled person would, apart from other treatments, use a tear substitute as protecting agent

in many of these conditions, see in this sense D3, cited pages, supra.

For the analysis of the inventive step of claims 42 and 45-47, D1 is considered to be the closest prior art. The difference between the present application and D1 is that in the present application HDL and Apolipoproteins are used in the preparation of pharmaceutical compositions for treating the conditions set forth in claim 3. In view of this difference the problem solved by the present application can be defined as the provision of alternative pharmaceutical compositions for treating eye disorders.

42 45-47 NOV

There is no suggestion in the prior art concerning the use of said compounds or compositions in the treatment of the said diseases and thus the subject matter of these claims involves an inventive step in the sense of Article 33(3) PCT.

For the assessment of the presently worded claims 1-18 and 37-47 on the question whether their subject matter is industrially applicable, no unified criteria exist in the PCT. The patentability under national patent laws can also be dependent on the formulation of the claims. The EPO, for example, does not recognise the subject matter of claims to the use of a compound in medical treatment as being industrially applicable, but will allow, however, claims to a known compound for first medical use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The Applicant is requested to bear in mind that according to Rule 66.4 PCT the issuance of an additional written opinion is facultative. Moreover, as the final action in the PCT-II procedure is an International Preliminary Examination Report and not a decision a violation of the right to be heard cannot exist. The Applicant cannot therefore rely on obtaining a second written opinion before the International Preliminary Examination Report is issued and is requested to answer to this first written opinion in a complete manner.

VII. Certain defects in th international application

Th following defects in the form or contents of the international application hav been noted:

The IPEA has received the description pages with the hand-written amendments. However, the amended typed sheets pages 4 and 6 of the description as filed with letter of 19-07-1998 are missing or have not been received. The Applicant is kindly requested to provide the IPEA with said amended typed pages 4 and 6.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The definition of the diseases as "disorders of the anterior segment of the eye, manifested by mechanical or chemical damage" (claims 1, 37) encompasses a high number of ill-defined malfunctions of the eye, as a result of which the scope of protection of said claims is not well defined, is interpretable and thus the said claims 1 and 37 are unclear in the sense of Article 6 PCT.

Claims 1-8 and 47-54 lack support in the sense of Article 6 PCT, see above item V.

Paragraph ii) of claims 6, 24 and 42 is unclear. It is vague and thus unclear what is meant by "at least one other lipid component of HLD other than cholesterol and cholesteryl-ester".

Lipofundin and Intralipid are trade marks. The said expressions do not convey any technical information for the skilled person and are therefore unclear, see Guidelines, C-III, 4.5b.

Claims 46-47 should be dependent of claim 42 (not 41 as it is written)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/17, 31/66, A01N 1/00

A1

(11) International Publication Number: **WO 98/30233**

(43) International Publication Date: 16 July 1998 (16.07.98)

(21) International Application Number: PCT/IL98/00012

(22) International Filing Date: 13 January 1998 (13.01.98)

(30) Priority Data:
120005 14 January 1997 (14.01.97) IL

(71) Applicant *(for all designated States except US)*: RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH & INDUSTRIAL DEVELOPMENT LTD. [IL/IL]; P.O. Box 39296, 61392 Tel Aviv (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SAVION, Naphtali [IL/IL]; Oranim Street 9/16, 54052 Givat Shmuel (IL). SOLOMON, Arieh [IL/IL]; Lipsky Street 23, 62105 Tel Aviv (IL).

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF THE EYE

(57) Abstract

A pharmaceutical composition for the treatment of disorders of the anterior segment of the eye or for the preservation of isolated cornea. The composition comprises, as an active ingredient, an agent capable of causing a net efflux of cholesterol from cells.

PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF THE EYE

FIELD OF THE INVENTION

The present invention concerns pharmaceutical compositions for the treatment of the eyes and more specifically for the treatment of disorders of the anterior segment of the eye.

BACKGROUND OF THE INVENTION

The protective structures of the anterior surface of the eye include the eyelids, conjunctiva, and the cornea. The posterior surfaces of the lids are covered with a mucous membrane and the palpebral conjunctiva which reflects onto the eye to become the bulbar conjunctiva. The bulbar conjunctival epithelium is continuous with the corneal epithelium which accounts for about 10% of the anterior surface of the eye and is where most of the stationary refraction occurs.

The corneal epithelium is 4-5 cells thick and the superficial cells contain many microvilli. These aid in maintaining the moisture of the epithelial surface by promoting the adhesion of the tear film to the surface. This film lubricates the anterior surface of the eye to decrease the frictional forces arising from the persistent blinking movements of the eyelids, foreign particles on the surface of the eye, and the rotational movements of the eyeball. The tear film also transfers oxygen from ambient air to the cornea.

The anterior surface of the eye is vulnerable to damages inflicted by various causes including mechanical abrasion of the cornea; contact lens wearing; spontaneous peeling of the epithelium; damaged epithelium and stroma following photo-refractive keratectomy; chemical burns; over exposure to ultraviolet light including sunlight; systemic diseases such as Sjogren syndrome, Steven-Johnson syndrome, Cicatricial pemphigoid syndrome; chronic edema of cornea with recurrent erosion of epithelium; impaired tear film formation, and conditions following damage of epithelia due to radial keratotomy.

Aging often causes disorders resulting from slow regeneration of the epithelium. The impaired regeneration and abnormality of the cells causes thinning of the epithelial layer and its impaired adherence to the basal lamina thus decreasing the ability of the cornea to retain the tear film leading to further epithelial damage.

Following injury to the corneal epithelium, nearby cells retract slightly, round up and begin an ameboid migration from the basal layer across the exposed basement membrane to cover the defect with a new monolayer of cells. These cells then take on the characteristics of a new basal layer and undergo mitosis to gradually fill in the defect with the full complement of four to five layers of cells. Present treatment for corneal wounds involves applying eye drops to the surface in order to protect the delicate healing process from erosion due to blinking and the other sources of friction. There are no currently used medicaments that promote the healing process itself. Attempts to administer fibronectin in order to promote healing of persistent defects of the corneal epithelium failed (Fukuda *et al., Am J. Ophthalmol.*, **119**(3):281-287, (1995)).

It would have been highly desirable to provide an ophthalmic composition capable of protecting the corneal epithelium and enhancing its healing and regeneration.

The rate of cell proliferation in many cell types has been correlated with the rate of cholesterol synthesis, and more specifically with the biosynthesis

of various intermediates in the cholesterol biosynthesis pathway and their by-products such as farnesylated proteins and others. Thus, inhibition of an early enzyme in the biosynthesis of cholesterol inhibits cell growth in cultured fibroblasts (McGuire *et al., J. Biol. Chem.*, **268**:22227-22230, (1993)). Factors which cause cholesterol efflux from cells (e.g. high density lipoproteins, HDL) alleviate the negative feedback inhibition of cholesterol synthesis and enhance growth of MDCK cells *in vitro* (Gospodarowicz *et al., J. Cell. Physiol.*, **117**:76-90, (1983)).

The cornea is an avascular organ obtaining nutrition from the vasculature of the limbus by diffusion. At the outer surface of the cornea, the epithelium is essentially isolated from the plasma's large complexes such as HDL which hardly diffuse through the cornea. Thus, HDL which performs the *"reverse cholesterol transport"* from peripheral organs to the liver (Glomset, J.A., *J. Lipid Res.*, **9**:155-167, 1968) is unable to perform this task in the corneal epithelium.

SUMMARY OF THE INVENTION

The present invention is based on the surprising finding that high density lipoprotein (HDL), or a combination of its non-cholesterol lipid constituents (phospholipids, and other lipids such as triglycerides and glycerol), which are capable of forming reconstituted HDL, promotes normal healing and regeneration of damaged eye epithelium.

Both HDL and said lipid constituents were able to initiate the process of healing, to increase its rate, and to promote reversion of the damaged epithelium of the eye to the normal state, i.e. where the damaged area is covered again by layers of epithelial cells.

Thus, the present invention concerns a pharmaceutical composition for the treatment of disorders of the anterior segment of the eye comprising, as an

active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an opthalgestically acceptable carrier.

The term *"treatment"* refers to curing of the disorder of the eye, to alleviation of some of the undesired symptomes of various eye disorders, and/or to prevention of various eye disorders before they are manifested.

The term *"anterior segment of the eye"* refers to the corneal and conjunctival epithelium and includes the epithelial cells, as well as the glands present in the epithelium.

The term *"disorders of the anterior segment of the eye"* refers to disorders which cause physical damage to the corneal or conjunctival epithelium, to disorders which decrease the rate of regeneration of cells making up this epithelium, or to disorders causing diminished secretions from glands present in the conjunctival epithelium, or to a combination of some of these disorders.

Typical disorders of the anterior segment of the eye caused by physical or chemical damage are: mechanical abrasion of the cornea, corneal epithelial defects created by wearing contact lenses, corneal epithelial defects created by spontaneous peeling of the epithelium, corneal damage following photo-reactive keratectomy, injuries caused by chemical substances, damage caused by exposure to ultraviolet light, systemic diseases creating damage to the corneal epithelium and conjunctiva, for example, Sjorgren-Syndrome, Steven-Johnson Syndrome, Cicatricial Pemphigoid Syndrome, chronic edema of the cornea with recurrent erosion of epithelium and the like.

Typical disorders of the anterior segment of the eye caused by a decrease in the rate of generation of cells include deterioration of the eye due to old age or an anti-proliferative treatment.

Typical disorders of the anterior segment of the eye caused by diminished secretion are dry eye and tear film dysfunction due to old age, various diseases or as side effects of systemic medication.

The pharmaceutical composition of the present invention may be administered to persons suffering from disorders which cause damage to the corneal or conjunctival epithelia, or in conjunction with treatments which are known to cause such damage, for example, laser or radial keratectomy or administration of various systemic or topical medications.

The pharmaceutical compounds of the invention, due to their amphoteric nature, are also capable of substituting for the natural tear film and may serve as lubricants, and thus, beyond their effect in the promotion of healing of the damaged eye epithelium, are also capable of relieving the symptoms of dry eye. Dry eye symptomes may be the cause of the physical damage of the epithelium or the result of it, or may be the sole disorder affecting the person's eye. Where the pharmaceutical composition is intended, *inter alia*, to treat dry eye, it should also include water/saline and a viscous substance such as an ophthalmologically acceptable polysaccharide in order to mimic as closely as possible the constituants of natural tears.

The active agents of the invention are those capable of causing a net efflux of cholesterol from cells. Locating candidate agents capable of generating a net cholesterol efflux, may be carried out, for example, by determining the net efflux of labeled cholesterol from cells according to the method described in Naphtali Savion and Shlomo Kotev-Emeth, *Eur. J. Biochem.*, **183**:363-370 (1989). Briefly, confluent endothelial or smooth muscle cultures are allowed to incorporate H^3-cholesterol. The candidate to be tested as an effector of cholesterol efflux is then added to the cell culture and the percentage of radioactivity remaining in the cells after 24 hrs. is determined. Candidates which are able to significantly lower the amount of labeled cholesterol in these cells, are those which are capable of serving as active agents in the pharmaceutical compositions of the invention.

Preferably, the present invention concerns a pharmaceutical composition for the treatment of disorders of the anterior segment of the eye

comprising as an active agent at least one compound selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or spingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester; and

iv. at least one HDL Apolipoprotein.

The term *"high density lipoprotein"* refers to lipoproteins which may be isolated from humans or other mammalian sources (e.g. bovine plasma), for example, as specified in Denis Gospodarowicz *"Methods for Preparation of Media, Supplements, and Substrata for Serum-Free Animal Cell Culture"*, pp. 69-86, 1984, Alan R. Liss, Inc., New York, New York or other isolation methods based on the density of the HDL.

The term *"phospholipids"*, refers to phospholipids which naturally occur in HDL such as phosphatidylcholine, phosphatidylserine and phosphatidylinositol. An example of *"sphingolipids"* is sphingomyelin.

The term *"and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester"* refers to glycerides, glycerol and triglycerides. In accordance with the invention glycerides and triglycerides which are not present naturally in HDL, but have an analogous function to glycerides and triglycerides present in HDL may also be used. The composition of matter comprising the non-cholesterol and the non-cholesteryl-ester lipid components of HDL (generally phospholipids, triglycerides and glycerides) is termed *"reconstituted HDL"* (Gillote *et al., J. Biol. Chem.*, **271**:23792-23798, 1996). This term refers to a complex comprising phospholipids, triglycerides and glycerides, which differs from natural HDL by the absence of cholesterol, cholesteryl-esters, and apolipoproteins.

Reconstituted HDL particles are prepared by the chelate dispersion/Bio-Bead removal technique (Sparks *et al., J. Biol. Chem.* **267**:25830-25838, 1992). Typically, compounds which are used in intravenous nutrition as a source of essential fatty acids, are suitable for serving as the lipid components of HDL. Example being Intralipid™, (Pharmacia AB, Sweden), Lipofundin™ (Braun Melsungen AG, Germany) and others.

The term *"HDL apolipoproteins"* refers typically to apolipoprotein A-I, A-IV and E-apolipo-proteins or a combination thereof, either isolated from a human or mammalian source (Savion and Gamliel, *Arteriosclerosis*, **8**:178-186, 1988). Apolipoprotein-E is purified according to Wernette-Hammond *et al., J. Biol. Chem.*, **264**:9094-9101, 1989. The HDL apolipoproteins may also be prepared by various genetic engineering methods described in Breslow, *et al., Proc. Natl. Acad. Sci.*, **79**:6861-6865, 1982. For example: Human Apolipoprotein A-I gene can be prepared according to the method of Karathanasis *et al., Proc. Natl. Acad. Sci.*, **80**:6147-6151, 1983; Human Apolipoprotein A-IV gene according to Elshourbagy, *et al., J. Biol. Chem.*, **262**:7973-7981, 1987; and Human Apolipoprotein E gene according to Das, *et al., J. Biol. Chem.*, **260**: 6240-6247, 1985; Paik, *et al., Proc. Natl. Acad. Sci.*, **82**:3445-3449, 1985.

The composition of the present invention may further comprise albumin.

Albumin is the most abundant plasma protein and serves as the plasma carrier of free fatty acids. Each albumin molecule has 27 binding sites for fatty acids. Albumin may thus serve as a scavenger for toxic free fatty acids released by damaged anterior chamber tissue included in reconstituted HDL.

The pharmaceutical compositions of the invention may further comprise other ingredients having ophthalgesic affects, especially those which are known to facilitate healing and regeneration of cornea and conjunctiva such as growth factors, for example, keratinocyte growth factor (KGF/FGF7), or

epidermal growth factor (EGF) and other growth factors of the EGF family known in the art; various attachment factors such as laminin or fibronectin, and extracellular matrix components such as collagen, heparan sulfate proteoglycans and others.

The pharmaceutical compositions of the invention may also include agents capable of providing ultraviolet light protection, such as oxybenzone 3%, and other such preparations known in the art.

The pharmaceutical compositions of the invention should be administered in the form of eye drops or eye salves together with opthalgestically acceptable carriers. The composition may be in the form of an emulsion, micelles liposomes, etc. The concentration of the active ingredients in the composition should be in the range of 0.1-20%, preferably 0.2-10%, most preferably 0.2-2%.

Some disorders of the eye that are to be treated by the pharmaceutical compositions of the invention include diminished liquid clearance from the eye causing water retention which eventually leads to the rupture of the eye membranes. In such cases, it is preferable that the compositions of the invention be presented in a hyperosmotic formulation which can serve to draw excess liquid from the eye. Such hyperosmotic formulation may be formed, for example, by the addition of NaCl to the composition.

By another aspect, the invention comprises a method for the treatment of disorders of the anterior segment of the eye comprising administering to a subject in need of such treatment at least one agent capable of causing a net efflux of cholesterol from cells.

By another aspect, the invention comprises use of at least one agent capable of causing a net efflux of cholesterol from cells for the preparation of a medicament for the treatment of disorders of the anterior segment of the eye.

By yet another aspect, the present invention concerns a storage preparation for storing and maintaining isolated corneas, for example, in an eye

bank. In order to maintain the viability of epithelial cells as well as the exposed endothelium of the eye, it is preferable to add to the storage medium an effective amount of at least one agent capable of causing a net efflux of cholesterol from cells, as explained above.

The invention now will be illustrated with reference to some non-limiting drawings and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows fluorescein staining of untreated damaged eye cornea 5 days after surgery (x 16);

Fig. 2 shows fluorescein staining of untreated damaged eye cornea 12 days after surgery (x 16);

Fig. 3A shows the histological appearance of normal cornea;

Fig. 3B shows the histological appearance of untreated damaged eye cornea 12 days after surgery;

Fig. 4 shows fluorescein staining of damaged eye cornea following Intralipid™ treatment;

Fig. 5 shows the histological appearance of damaged eye cornea following Lyteers™ treatment;

Fig. 6 shows fluorescein staining of damaged eye cornea after 3 days of treatment with HDL;

Fig. 7 shows fluorescein staining of damaged eye cornea at the end of treatment with HDL;

Fig. 8 shows the the histological appearance of damaged eye cornea after HDL treatment;

Fig. 9 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lipofundin™; and

Fig. 10 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lyeteers™.

DETAILED DESCRIPTION OF THE INVENTION

I. Experimental Procedures

A. Animal model of corneal epithelium and conjunctival epithelium damage

A rabbit model for keratoconjunctivitis (Gilbard *et al., J. Inv. Ophthal. Vis. Sci.*, 2:225-228 (1987)) was used with slight modification. Surgery performed on anesthetized rabbits using a surgical microscope (Inami, Japan) involved excision of the plical fold over the eye, occlusion of the lacrimal duct, and peeling of the palpebral and bulbar conjunctiva. This surgery was done on one eye of 20 rabbits of average age 3 months of both sexes. Surgery and all subsequent treatments were done in accordance with ARVO rules for animal care in research. Tear film osmolarity is elevated by postoperative day 1. Corneal epithelial glycogen levels decline progressively, and conjunctival goblet cell density decreases. These pathologies lead to corneal epithelium damage covering the entire corneal surface by the fifth postoperative day, and it was at this time that treatment of the eyes commenced.

B. HDL preparation

HDL was prepared from human plasma by differential ultracentrifugation flotation (*Havel et al.*, "Distribution and chemical composition of ultracentrifugally lipoproteins in human serum", *J. Clin. Invest.*, 34:1345-53, (1995)).

C. Evaluation of Rabbit Cornea

Lesions in fluorescein stained corneas were clinically evaluated by biomicroscopy using a slit lamp (Haag Streit, Switzerland) with cobalt filter illumination. Photography of the fluorescein staining was taken with a slit lamp mounted camera (Topcon, Japan). At the end of each experiment, the rabbits were

sacrificed and the cornea were excised, fixed in paraformaldehyde and examined for epithelial lesions.

D. Histological examination

At the end of the treatment, the rabbits were sacrificed by a lethal dose of intravenously injected pentobarbitone. The eyes were enuclated and the corneas fixed in 4% paraformaldehyde. Corneas were embedded in paraffin blocks, sectioned, and with hematoxylin-eosine for light microscope examination.

II. Treatment of corneal epithelium damage caused by conjunctival epithelium damage

Example 1

Rabbits with cornea damage induced as above were treated as follows: Five rabbits were treated with commercially available artificial tears (Lyteers™), five rabbits were treated with HDL (1mg protein/ml) in phosphate buffered saline, five rabbits were treated with a commercially available lipid mixture (10% Intralipid™: 10% soybean oil, 1.2% egg phospholipids, 2.2% glycerol), and two rabbits were left untreated. Treatment consisted of applying two drops to the eye 3 times a day for seven consecutive days. The eyes were evaluated clinically during the experiment and pictures were taken every other day of the fluorescein stained corneas.

Fluorescein staining of damaged eyes 5 and 12 days following surgery is shown in Figs. 1 and 2, respectively. As can be seen, the surface of the untreated eye became progressively more scratched and opaque with time leading eventually to blurred vision.

Histological staining of damaged eye is shown in Fig. 3B and is compared to histological staining of normal eye 3A. As can be seen, in the damaged eye there has been complete erosion of the exposed epithelium due to

persisant rubbing of the epithelium by the lids as well as severe keratitis and vascularization of the cornea.

Animal eyes that were treated with Intralipid™ show complete reversal to normal morphological structure as indicated by fluorescein staining (Fig. 4). However, animal eyes treated with Lyteer™, (Fig. 5) (commercially available artifical tears composed mainly of water and a viscous substance) did not return to normal. In contrast to normal eyes, the damaged area did not become covered again with normal layers of cuboidal epithelial cells and a single top layer of wing cells, but was covered instead by only a single layer of wing cells. These results indicate that artificial tears such as Lyteer™ cannot promote regeneration of normal eye epithelium.

In contrast to this, animal eyes treated with HDL showed essentially a complete return to normal morphological structure as indicated by fluorescein staining taken on the third day (Fig. 6) and at the end of treatment (Fig. 7) as well as by histological staining (Fig. 8). Histological staining shows essentially a complete return to normal of the eye epithelium characterized by formation of several layers of cuboidal cells and a single top layer of wing cells.

These results clearly indicate that both HDL and Intralipid™ are able to promote healing and regeneration of damaged eye epithelium and return to normal epithelium.

Example 2

Three rabbits underwent central corneal peeling of the epithelium in both eyes. The area of peeling, 8 mm in diameter, was first demarcated with trephine, and the epithelium excised with a scalpel. The right eye of each rabbit was treated with two drops of Lipofundin™ 10% three times a day, while the left eye was treated with the same dose of Lyeteers™. The cornea were stained with fluorescein and photographed immediately after epithelium removal and on the third and fifth day afterwards. On the seventh day, the rabbits were sacrificed by

a lethal dose of pental and the corneas were excised, fixed and stained for light microscopy. The denuded area in the fluorescein and fixed corneas was determined, and the extent of remaining damage calculated as the remaining denuded area divided by the initial denuded area. The results appear in Table 1 below which gives the fraction of initial damage remaining 0, 3, 5, and 7 days after peeling the epithelium. The results show that the rate of healing was faster in the Lipofundin™ treated eyes. Figs. 9 and10 show the epithelium of a Lipofundin™ treated eye and a treated eye, respectively, after 7 days of treatment. Whereas the Lipofundin™ appears close to, in the Lyeteers™ treated eye the epithelium still appears thin, immature, and containing only flat and wing cells which have migrated to the denuded area.

Table 1

	Treatment	
Time (Days)	**Lipofundin™**	**Lyeteers™**
0	1.00	1.00
3	.304	.378
5	.036	.107
7	0	0

III. Clinical study on the effect of Lipofundin™ on dry eye

Experimental

Eight female patients suffering from dry eye and presenting less than 10 mm of wet strip in a Shirmer test, were included in this study. The patients ranged in age from 62 to 74 years.

Each patient received a bottle of Lipofundin™ 5% and was instructed to instill two drops in one eye three times a day for one week, while continuing the

previous treatment in the other eye. Each patient was asked to fill out a questionnaire before and after the week of treatment in which she was to rank the following parameters on a scale of 0 (no complaint) to 4 (very severe): itching, tearing, a sensation of "sand" under the upper eyelid, and photophobia. The appearance of the anterior segments of the eyes was evaluated clinically for redness, inflammation, and discharge from the conjunctival sac.

Results

The results of the clinical studies are given in Table 2. All of the patients reported a general improvement in their symptoms and asked to continue treatment with Lipofundin™ for a longer period of time. Seven of the eight patients had no complaints about the treatment. One patient, presenting the most severe case of dry eye at the beginning of the study, complained of a burning sensation lasting about two minutes following the instillation of Lipofundin™ during the first two days of treatment. Lipofundin™ was thus well tolerated by the patients and ameliorates symptoms in people suffering form dry eye.

Table 2

	Before Treatment						After Treatment						
Degree Symptom	0	1	2	3	4	Average ± SD	0	1	2	3	4	Average ± SD	p value
Itching		1	3	3	1	2.50 ± 0.93	4	4				0.50 ± 0.53	0.000057
Tearing			3	2	3	3.00 ± 0.92	5	3				0.37 ± 0.51	0.000031
Sand		1	4	3		2.25 ± 0.71	6	2				0.25 ± 0.46	0.000051
Photophobia			1	4	3	3.25 ± 0.71	5	2	1			0.50 ± 0.76	0.000014

CLAIMS:

1. A pharmaceutical composition for the treatment of disorders of the anterior segment of the eye comprising, as an active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an ophthagestically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

3. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

4. A pharmaceutical composition according to Claim 3, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

5. A pharmaceutical composition according to Claim 1, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

6. A pharmaceutical composition according to Claim 5, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

7. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

8. A pharmaceutical composition according to Claim 7, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

9. A pharmaceutical composition according to Claims 1 to 8, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

10. A pharmaceutical composition according to Claims 1 to 8 wherein said agent is Lipofundin™

11. A pharmaceutical composition according to Claims 1 to 8 wherein said agent is Intralipid™

12. A pharmaceutical composition according to Claims 9-11, further comprising albumin.

13. A pharmaceutical composition according to Claim 9, wherein the HDL is human or bovine HDL.

14. A pharmaceutical composition according to Claim 9, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

15. A pharmaceutical composition according to Claim 9, wherein the sphingolipids are sphingomyelins.

16. A pharmaceutical composition according to Claim 9, wherein the other lipid components of HDL are triglycerides and/or glycerol.

17. A pharmaceutical composition according to Claim 9, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I,

Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

18. A pharmaceutical composition according to Claims 1 to 17, further comprising a growth factor, an attachment factor or an extracellular component.

19. A pharmaceutical composition according to Claim 18, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

20. A pharmaceutical composition according to Claim 18, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

21. A pharmaceutical composition according to Claim 18, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

22. A pharmaceutical composition according to any one of Claims 1 to 21 further comprising an agent capable of providing protection from U.V. radiation.

23. A pharmaceutical composition according to Claim 22, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

24. A method for the treatment of disorders of the anterior segment of the eye comprising administering to a subject in need of such treatment at least one agent capable of causing a net efflux of cholesterol from cells.

25. A method according to Claim 24, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

26. A method according to Claim 24, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

27. A method according to Claim 26, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

28. A method according to Claim 24, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

29. A method according to Claim 28, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

30. A method according to Claim 24, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

31. A method according to Claim 30, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

32. A method according to Claims 24 to 31, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

33. A method according to Claims 24 to 31 wherein said agent is Lipofundin™

34. A method according to Claims 24 to 31 wherein said agent is Intralipid™

35. A method according to Claims 32-34, further comprising administering albumin.

36. A method according to Claim 32, wherein the HDL is human or bovine HDL.

37. A method according to Claim 32, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

38. A method according to Claim 32, wherein the sphingolipids are sphingomyelins.

39. A method according to Claim 32, wherein the other lipid components of HDL are triglycerides and/or glycerol.

40. A method according to Claim 32, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

41. A method according to Claims 24 to 40, further comprising a growth factor, an attachment factor or an extracellular component.

42. A method according to Claim 41, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

43. A method according to Claim 41, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

44. A method according to Claim 41, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

45. A method according to any one of Claims 24 to 44 further comprising administering an agent capable of providing protection from U.V. radiation.

46. A method according to Claim 45, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

47. Use of at least one agent capable of causing a net efflux of cholesterol from cells for the preparation of a medicament for the treatment of disorders of the anterior segment of the eye.

48. A use according to Claim 47, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

49. A use according to Claim 47, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

50. A use according to Claim 49, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

51. A use according to Claim 47, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

52. A use according to Claim 51, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

53. A use according to Claim 47, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

54. A use according to Claim 53, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

55. A use according to Claims 47 to 54, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

56. A use according to Claims 47 to 54 wherein said agent is Lipofundin™

57. A use according to Claims 47 to 54 wherein said agent is Intralipid™

58. A use according to Claim 54, wherein the HDL is human or bovine HDL.

59. A use according to Claim 54, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

60. A use according to Claim 54, wherein the sphingolipids are sphingomyelins.

61. A use according to Claim 54, wherein the other lipid components of HDL are triglycerides and/or glycerol.

62. A use according to Claim 54, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

63. A storage medium for the preservation of isolated cornea comprising at least one agent capable of causing net efflux of cholesterol from cells.

64. A storage medium according to Claim 63, wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

65. A storage medium according to Claim 63, wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. Lipofundin™

ii. Intralipid™

66. A storage medium according to Claims 64 and 65, further comprising albumin.

67. A storage medium according to Claim 64, wherein the HDL is human or bovine HDL.

68. A storage medium according to Claim 64, wherein the phospho-lipids are selected from the group consisting of: phosphatidylcholine. phosphatidylethanolamine, phosphatidylserine and phosphatidylinosital.

69. A storage medium according to Claim 64, wherein the sphingolipids are sphingomyelin.

70. A storage medium according to Claim 64, wherein the other lipid components of HDL are triglycerides and/or glycerol.

71. A storage medium according to Claim 64, wherein the apolipoprotein is selected from the group consisting of Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

FIG. 1

FIG. 2

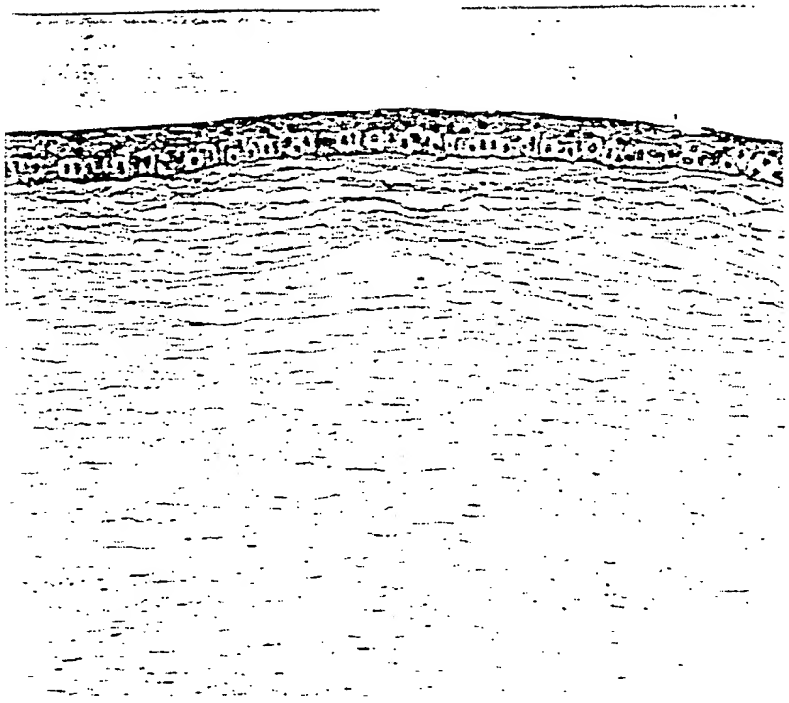

FIG.3A

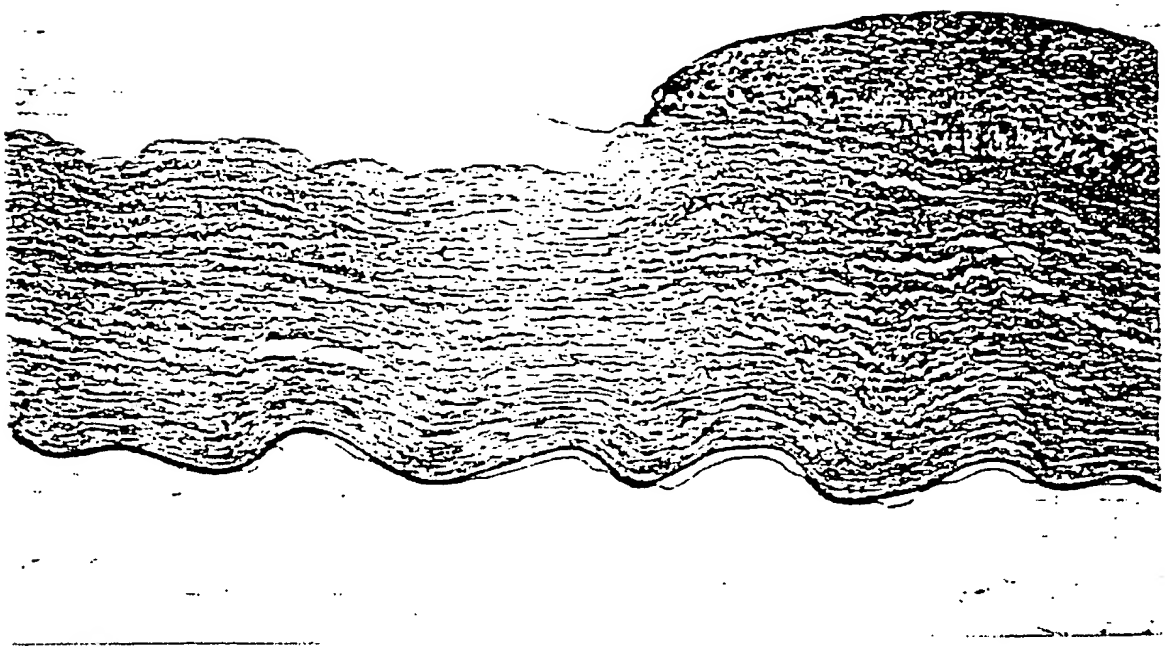

FIG. 3B

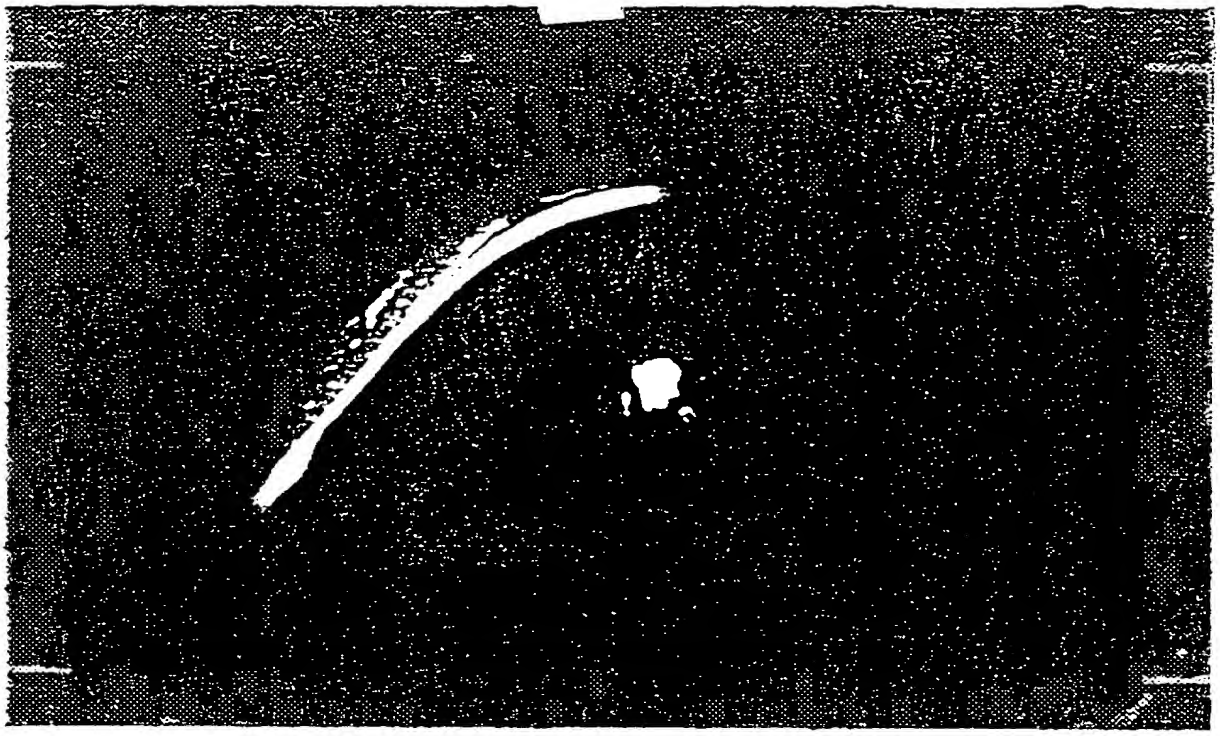

FIG. 4

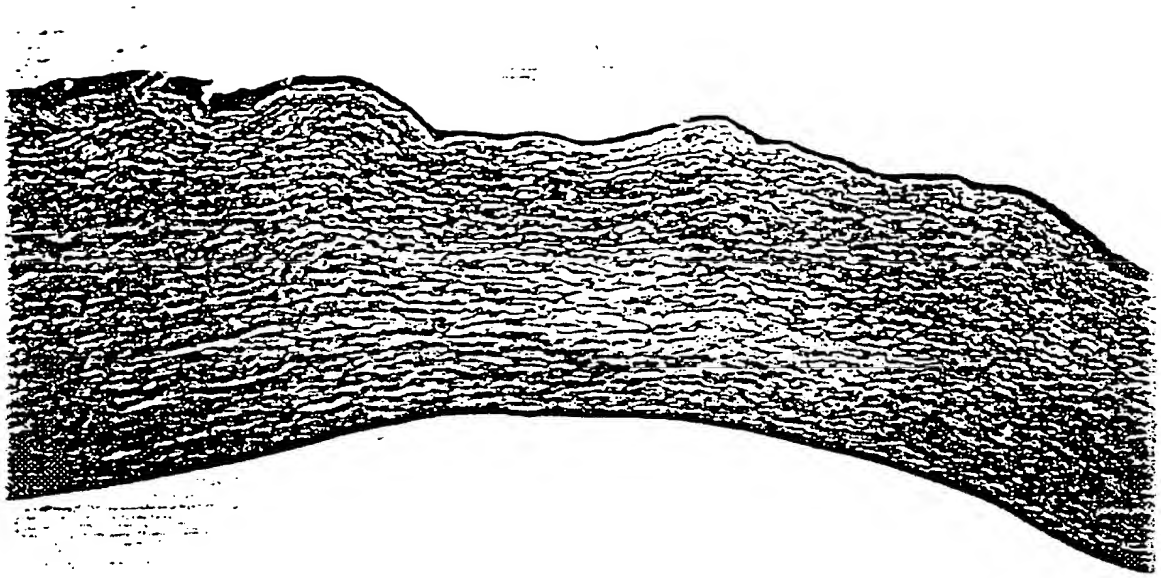

FIG. 5

FIG. 6

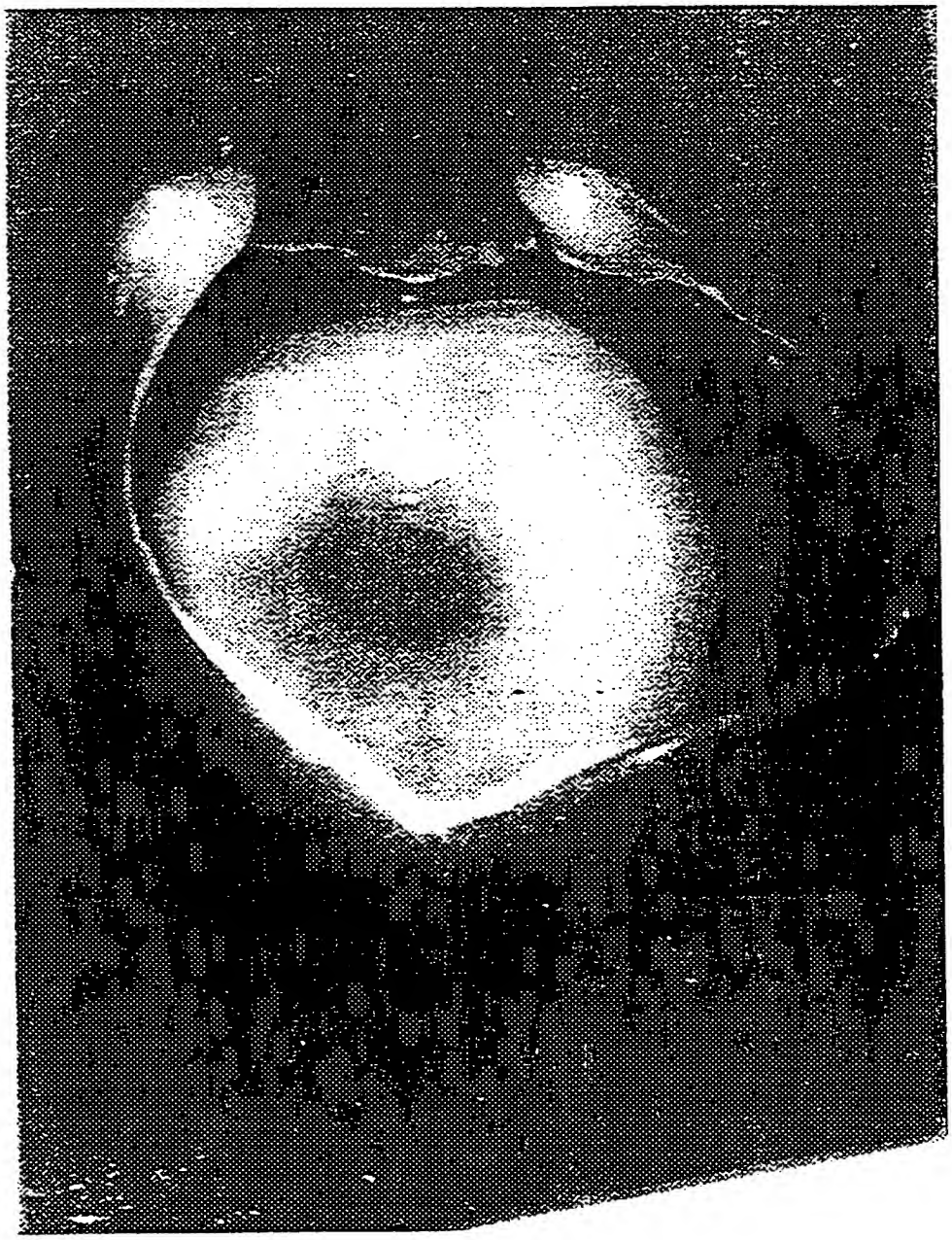

FIG. 7

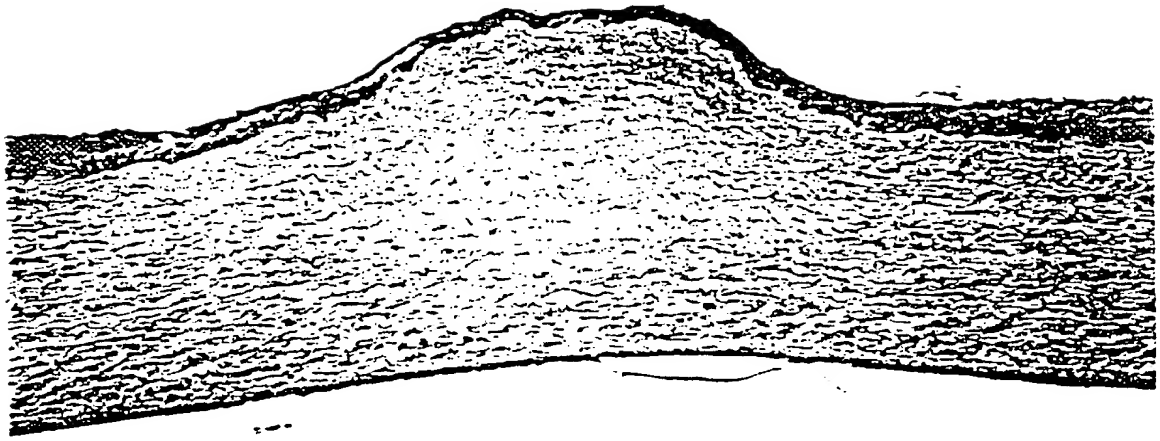

FIG. 8

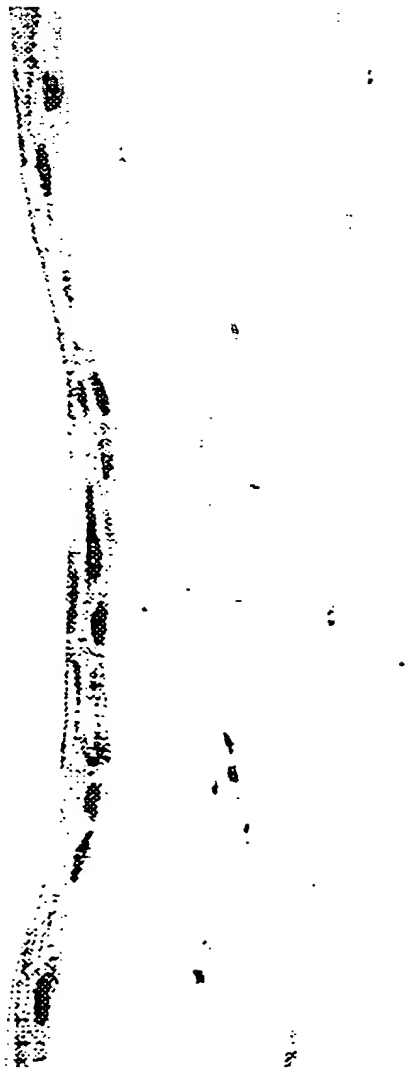

FIG. 9

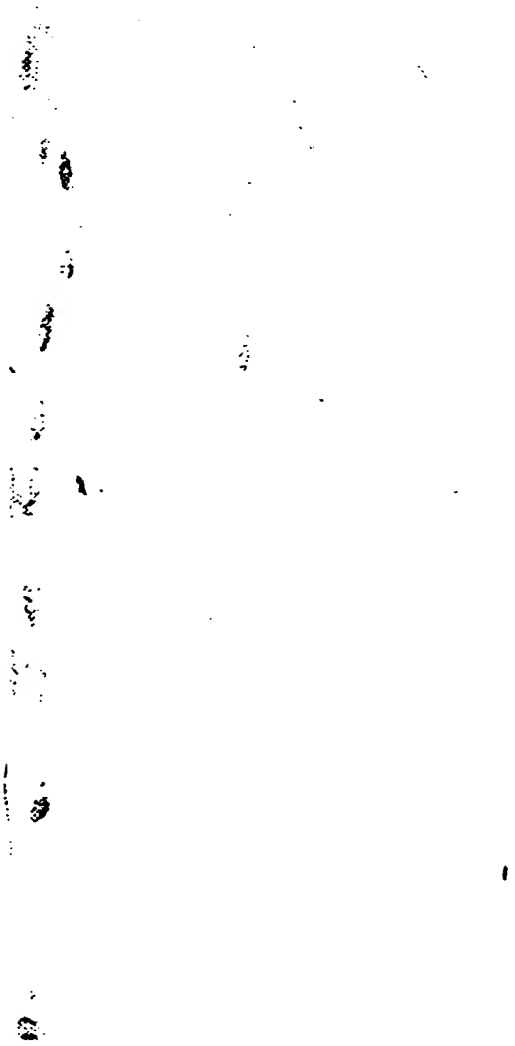

FIG. 10

active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an opthalgestically acceptable carrier.

The term *"treatment"* refers to curing of the disorder of the eye, to alleviation of some of the undesired symptomes of various eye disorders, and/or to prevention of various eye disorders before they are manifested.

The term *"anterior segment of the eye"* refers to the corneal and conjunctival epithelium and includes the epithelial cells, as well as the glands present in the epithelium.

The term *"disorders of the anterior segment of the eye"* refers to disorders which cause physical damage to the corneal or conjunctival epithelium, to disorders which decrease the rate of regeneration of cells making up this epithelium, or to disorders causing diminished secretions from glands present in the conjunctival epithelium, or to a combination of some of these disorders.

Typical disorders of the anterior segment of the eye caused by physical or chemical damage are: mechanical abrasion of the cornea, corneal epithelial defects created by wearing contact lenses, corneal epithelial defects created by spontaneous peeling of the epithelium, corneal damage following photo-reactive keratectomy, injuries caused by chemical substances, damage caused by exposure to ultraviolet light, systemic diseases creating damage to the corneal epithelium and conjunctiva, for example, Sjorgren-Syndrome, Steven-Johnson Syndrome, Cicatricial Pemphigoid Syndrome, chronic edema of the cornea with recurrent erosion of epithelium and the like.

Typical disorders of the anterior segment of the eye caused by a decrease in the rate of generation of cells include deterioration of the eye due to old age or an anti-proliferative treatment.

The pharmaceutical composition of the present invention may be administered to persons suffering from disorders which cause damage to the corneal or conjunctival epithelia, or in conjunction with treatments which are known to cause such damage, for example, laser or radial keratectomy or administration of various systemic or topical medications.

The active agents of the invention are those capable of causing a net efflux of cholesterol from cells. Locating candidate agents capable of generating a net cholesterol efflux, may be carried out, for example, by determining the net efflux of labeled cholesterol from cells according to the method described in Naphtali Savion and Shlomo Kotev-Emeth, *Eur. J. Biochem.*, 183:363-370 (1989). Briefly, confluent endothelial or smooth muscle cultures are allowed to incorporate ^{3}H-cholesterol. The candidate to be tested as an effector of cholesterol efflux is then added to the cell culture and the percentage of radioactivity remaining in the cells after 24 hrs. is determined. Candidates which are able to significantly lower the amount of labeled cholesterol in these cells, are those which are capable of serving as active agents in the pharmaceutical compositions of the invention.

Preferably, the present invention concerns a pharmaceutical composition for the treatment of disorders of the anterior segment of the eye comprising as an active agent at least one compound selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. a composition of matter termed *"reconstituted HDL"* and sphinogolipids comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester; and

iii. at least one HDL Apolipoprotein.

The term *"high density lipoprotein"* refers to lipoproteins which may be isolated from humans or other mammalian sources (e.g. bovine plasma), for example, as specified in Denis Gospodarowicz *"Methods for Preparation of Media, Supplements, and Substrata for Serum-Free Animal Cell Culture"*, pp. 69-86, 1984, Alan R. Liss, Inc., New York, New York or other isolation methods based on the density of the HDL.

The term *"phospholipids"*, refers to phospholipids which naturally occur in HDL such as phosphatidylcholine, phosphatidylserine and phosphatidylinositol. An example of *"sphingolipids"* is sphingomyelin.

The term *"and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester"* refers to glycerides, glycerol and triglycerides. In accordance with the invention glycerides and triglycerides which are not present naturally in HDL, but have an analogous function to glycerides and triglycerides present in HDL may also be used. The composition of matter comprising the non-cholesterol and the non-cholesteryl-ester lipid components of HDL (generally phospholipids, triglycerides and glycerides) is termed *"reconstituted HDL"* (Gillote *et al., J. Biol. Chem.*, 271:23792-23798, 1996). This term refers to a complex comprising phospholipids, triglycerides and glycerides, which differs from natural HDL by the absence of cholesterol, cholesteryl-esters, and apolipoproteins.

bank. In order to maintain the viability of epithelial cells as well as the exposed endothelium of the eye, it is preferable to add to the storage medium an effective amount of at least one agent capable of causing a net efflux of cholesterol from cells, as explained above.

The invention now will be illustrated with reference to some non-limiting drawings and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows fluorescein staining of untreated damaged eye cornea 5 days after surgery (x 16);

Fig. 2 shows fluorescein staining of untreated damaged eye cornea 12 days after surgery (x 16);

Fig. 3A shows the histological appearance of normal cornea;

Fig. 3B shows the histological appearance of untreated damaged eye cornea 12 days after surgery;

Fig. 4 shows fluorescein staining of damaged eye cornea following Intralipid™ treatment;

Fig. 5 shows the histological appearance of damaged eye cornea following Lyteers™ treatment;

Fig. 6 shows fluorescein staining of damaged eye cornea after 3 days of treatment with HDL;

Fig. 7 shows fluorescein staining of damaged eye cornea at the end of treatment with HDL;

Fig. 8 shows the histological appearance of damaged eye cornea after HDL treatment;

Fig. 9 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lipofundin™; and

Fig. 10 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lyeteers™.

persisant rubbing of the epithelium by the lids as well as severe keratitis and vascularization of the cornea.

Animal eyes that were treated with Intralipid™ show complete reversal to normal morphological structure as indicated by fluorescein staining (Fig. 4). However, animal eyes treated with Lyeteers™, (Fig. 5) (commercially available artifical tears composed mainly of water and a viscous substance) did not return to normal. In contrast to normal eyes, the damaged area did not become covered again with normal layers of cuboidal epithelial cells and a single top layer of wing cells, but was covered instead by only a single layer of wing cells. These results indicate that artificial tears such as Lyeteers™ cannot promote regeneration of normal eye epithelium.

In contrast to this, animal eyes treated with HDL showed essentially a complete return to normal morphological structure as indicated by fluorescein staining taken on the third day (Fig. 6) and at the end of treatment (Fig. 7) as well as by histological staining (Fig. 8). Histological staining shows essentially a complete return to normal of the eye epithelium characterized by formation of several layers of cuboidal cells and a single top layer of wing cells.

These results clearly indicate that both HDL and Intralipid™ are able to promote healing and regeneration of damaged eye epithelium and return to normal epithelium.

Example 2

Three rabbits underwent central corneal peeling of the epithelium in both eyes. The area of peeling, 8 mm in diameter, was first demarcated with trephine, and the epithelium excised with a scalpel. The right eye of each rabbit was treated with two drops of Lipofundin™ 10% three times a day, while the left eye was treated with the same dose of Lyeteers™. The cornea were stained with fluorescein and photographed immediately after epithelium removal and on the third and fifth day afterwards. On the seventh day, the rabbits were sacrificed by a lethal dose of pental and the corneas were excised, fixed and stained for light microscopy. The denuded area in the fluorescein and fixed corneas was determined, and the extent of remaining damage calculated as the remaining denuded area divided by the initial denuded area. The results appear in Table 1 below which gives the fraction of initial damage remaining 0, 3, 5, and 7 days after peeling the epithelium. The results show that the rate of healing was faster in the Lipofundin™ treated eyes. Figs. 9 and10 show the epithelium of a Lipofundin™ treated eye and Lyeteers™ treated eye, respectively, after 7 days of treatment. Whereas the Lipofundin™ appears close to normal, in the Lyeteers™ treated eye the epithelium still appears thin, immature, and containing only flat and wing cells which have migrated to the denuded area.

Table 1

	Treatment	
Time (Days)	**Lipofundin™**	**Lyeteers™**
0	1.00	1.00
3	.304	.378
5	.036	.107
7	0	0

5

CLAIMS:

1. A pharmaceutical composition for the treatment of disorders of the anterior segment of the eye comprising an ophthalgestically acceptable carrier and as an active ingredient an agent being high density lipoprotein (HDL).

2. A pharmaceutical composition according to Claim 1, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

3. A pharmaceutical composition according to Claim 1, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

4. A pharmaceutical composition according to Claim 1, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

5. A pharmaceutical composition according to Claim 4, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

6. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by a slow rate of regeneration of epithelial cells of the anterior segment of the eye.

7. A pharmaceutical composition according to Claim 6, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

8. A pharmaceutical composition for the treatment of diseases of the anterior segment of the eye selected from the group consisting of mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refructive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; recurrent erosion of epithelium; comprising an ophthalgestically acceptable carriers and as an active ingredient at least one agent selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids; and

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester.

9. A pharmaceutical composition according to Claim 8, wherein said agent is Lipofundin™

10. A pharmaceutical composition according to Claim 8, wherein said agent is Intralipid™

11. A pharmaceutical composition according to Claim 1 or 8, further comprising albumin.

12. A pharmaceutical composition according to Claim 1 or 8, wherein the HDL is human HDL, bovine HDL, or reconstituted HDL comprising of phospholipids and/or sphinolipids and at least one apolipoprotein.

13. A pharmaceutical composition according to Claim 8 or 12, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

14. A pharmaceutical composition according to Claim 8 or 12, wherein the sphingolipids are sphingomyelins.

15. A pharmaceutical composition according to Claim 8, wherein the other lipid components of HDL are triglycerides and/or glycerol.

16. A pharmaceutical composition according to Claim 12, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I and Apolipoprotein A-IV or a combination of both apolipoproteins.

17. A pharmaceutical composition according to Claims 1 to 8, further comprising a growth factor, an attachment factor or an extracellular matrix component.

18. A pharmaceutical composition according to Claim 17, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

19. A pharmaceutical composition according to Claim 17, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

20. A pharmaceutical composition according to Claim 17, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

21. A pharmaceutical composition according to Claims 1 to 8, further comprising an agent capable of providing protection from U.V. radiation.

22. A pharmaceutical composition according to Claim 21, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

23. A method for the treatment of disorders of the anterior segment of the eye comprising administering to a subject in need of such treatment a composition comprising an agent being high density lipoprotein (HDL).

24. A method according to Claim 23, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

25. A method according to Claim 23, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial

defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

26. A method according to Claim 23, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

27. A method according to Claim 26, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

28. A method according to Claim 23, wherein the disorders are manifested by a slow rate of regeneration of epithelial cells of the anterior segment of the eye.

29. A method according to Claim 28, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

30. A method for the treatment of diseases of the anterior segment of the eye selected from the group consisting of mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; recurrent erosion of epithelium comprising administering to a subject in need of such treatment at least one agent selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids; and

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester.

31. A method according to Claim 30, wherein said agent is Lipofundin™

32. A method according to Claim 30, wherein said agent is Intralipid™

33. A method according to Claim 23 or 30, further comprising albumin.

34. A method according to Claim 23 or 30, wherein the HDL is human HDL, bovine HDL, or reconstituted HDL comprising of phospholipids and/or sphinolipids and at least one apolipoprotein.

35. A method according to Claim 30 or 34, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol.

36. A method according to Claim 30 or 34, wherein the sphingolipids are sphingomyelins.

37. A method according to Claim 30, wherein the other lipid components of HDL are triglycerides and/or glycerol.

38. A method according to Claim 34, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I and Apolipoprotein A-IV or a combination of both apolipoproteins.

39. A method according to Claims 23 to 30, further comprising administering a growth factor, an attachment factor or an extracellular matrix component.

40. A method according to Claim 39, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

41. A method according to Claim 39, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

42. A method according to Claim 39, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

43. A method according to Claims 23 to 30, further comprising an agent capable of providing protection from U.V. radiation.

44. A method according to Claim 43, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

45. Use of high density lipoprotein (HDL) for the preparation of a medicament for the treatment of disorders of the anterior segment of the eye.

46. Use according to Claim 45, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

47. Use according to Claim 45, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

48. Use according to Claim 45, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

49. Use according to Claim 48, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

50. Use according to Claim 45, wherein the disorders are manifested by a slow rate of regeneration of epithelial cells of the anterior segment of the eye.

51. Use according to Claim 50, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

52. Use of at least one agent selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids; and

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester;

for the preparation of a medicament for treatment of disorders of the anterior segment of the eye selected from the group consisting of mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; with recurrent erosion of epithelium.

53. Use according to Claim 52, wherein said agent is Lipofundin™

54. Use according to Claim 52, wherein said agent is Intralipid™

55. Use according to Claim 45 or 52, wherein the HDL is human HDL, bovine HDL, or reconstituted HDL comprising of phospholipids and/or sphinolipids and at least one apolipoprotein.

56. Use according to Claim 52 or 55, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol.

57. Use according to Claim 52 or 55, wherein the sphingolipids are sphingomyelins.

58. Use according to Claim 52, wherein the other lipid components of HDL are triglycerides and/or glycerol.

59. Use according to Claim 55, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I and Apolipoprotein A-IV or a combination of both apolipoproteins.

60. A storage medium for the preservation of isolated cornea comprising at least one agent selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids; and

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester.

61. A storage medium according to Claim 60, wherein the agent is one or more of the group consisting of:

i. Lipofundin™

ii. Intralipid™

62. A storage medium according to Claims 60 and 61, further comprising albumin.

63. A storage medium according to Claim 60, wherein the HDL is human HDL, bovine HDL, or reconstituted HDL comprising of phospholipids and/or sphinolipids and at least one apolipoprotein.

64. A storage medium according to Claim 60 or 63, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinosital.

65. A storage medium according to Claim 60 or 63, wherein the sphingolipids are sphingomyelin.

66. A storage medium according to Claim 60, wherein the other lipid components of HDL are triglycerides and/or glycerol.

67. A storage medium according to Claim 63, wherein the apolipoprotein is selected from the group consisting of Apolipoprotein A-I and Apolipoprotein A-IV or a combination of both apolipoproteins.

active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an opthalgestically acceptable carrier.

The term *"treatment"* refers to curing of the disorder of the eye, to alleviation of some of the undesired symptomes of various eye disorders, and/or to prevention of various eye disorders before they are manifested.

The term *"anterior segment of the eye"* refers to the corneal and conjunctival epithelium and includes the epithelial cells, as well as the glands present in the epithelium.

The term *"disorders of the anterior segment of the eye"* refers to disorders which cause physical damage to the corneal or conjunctival epithelium, to disorders which decrease the rate of regeneration of cells making up this epithelium, or to disorders causing diminished secretions from glands present in the conjunctival epithelium, or to a combination of some of these disorders.

Typical disorders of the anterior segment of the eye caused by physical or chemical damage are: mechanical abrasion of the cornea, corneal epithelial defects created by wearing contact lenses, corneal epithelial defects created by spontaneous peeling of the epithelium, corneal damage following photo-reactive keratectomy, injuries caused by chemical substances, damage caused by exposure to ultraviolet light, systemic diseases creating damage to the corneal epithelium and conjunctiva, for example, Sjorgren-Syndrome, Steven-Johnson Syndrome, Cicatricial Pemphigoid Syndrome, chronic edema of the cornea with recurrent erosion of epithelium and the like.

Typical disorders of the anterior segment of the eye caused by a decrease in the rate of generation of cells include deterioration of the eye due to old age or an anti-proliferative treatment.

Typical disorders of the anterior segment of the eye caused by diminished secretion are dry eye and tear film dysfunction due to old age, various diseases or as side effects of systemic medication.

The pharmaceutical composition of the present invention may be administered to persons suffering from disorders which cause damage to the corneal or conjunctival epithelia, or in conjunction with treatments which are known to cause such damage, for example, laser or radial keratectomy or administration of various systemic or topical medications.

The pharmaceutical compounds of the invention, due to their amphoteric nature, are also capable of substituting for the natural tear film and may serve as lubricants, and thus, beyond their effect in the promotion of healing of the damaged eye epithelium, are also capable of relieving the symptoms of dry eye. Dry eye symptomes may be the cause of the physical damage of the epithelium or the result of it, or may be the sole disorder affecting the person's eye. Where the pharmaceutical composition is intended, *inter alia*, to treat dry eye, it should also include water/saline and a viscous substance such as an ophthalmologically acceptable polysaccharide in order to mimic as closely as possible the constituants of natural tears.

The active agents of the invention are those capable of causing a net efflux of cholesterol from cells. Locating candidate agents capable of generating a net cholesterol efflux, may be carried out, for example, by determining the net efflux of labeled cholesterol from cells according to the method described in Naphtali Savion and Shlomo Kotev-Emeth. *Eur. J. Biochem.*, **183**:363-370 (1989). Briefly, confluent endothelial or smooth muscle cultures are allowed to incorporate H^3-cholesterol. The candidate to be tested as an effector of cholesterol efflux is then added to the cell culture and the percentage of radioactivity remaining in the cells after 24 hrs. is determined. Candidates which are able to significantly lower the amount of labeled cholesterol in these cells, are those which are capable of serving as active agents in the pharmaceutical compositions of the invention.

Preferably, the present invention concerns a pharmaceutical composition for the treatment of disorders of the anterior segment of the eye

comprising as an active agent at least one compound selected from the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or spingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester; and

iv. at least one HDL Apolipoprotein.

The term *"high density lipoprotein"* refers to lipoproteins which may be isolated from humans or other mammalian sources (e.g. bovine plasma), for example, as specified in Denis Gospodarowicz *"Methods for Preparation of Media, Supplements, and Substrata for Serum-Free Animal Cell Culture"*, pp. 69-86, 1984, Alan R. Liss, Inc., New York, New York or other isolation methods based on the density of the HDL.

The term *"phospholipids"*, refers to phospholipids which naturally occur in HDL such as phosphatidylcholine, phosphatidylserine and phosphatidylinositol. An example of *"sphingolipids"* is sphingomyelin.

The term *"and at least one other lipid component of HDL other than cholesterol and cholesteryl-ester"* refers to glycerides, glycerol and triglycerides. In accordance with the invention glycerides and triglycerides which are not present naturally in HDL, but have an analogous function to glycerides and triglycerides present in HDL may also be used. The composition of matter comprising the non-cholesterol and the non-cholesteryl-ester lipid components of HDL (generally phospholipids, triglycerides and glycerides) is termed *"reconstituted HDL"* (Gillote *et al., J. Biol. Chem.*, 271:23792-23798, 1996). This term refers to a complex comprising phospholipids, triglycerides and glycerides, which differs from natural HDL by the absence of cholesterol, cholesteryl-esters, and apolipoproteins.

bank. In order to maintain the viability of epithelial cells as well as the exposed endothelium of the eye, it is preferable to add to the storage medium an effective amount of at least one agent capable of causing a net efflux of cholesterol from cells, as explained above.

The invention now will be illustrated with reference to some non-limiting drawings and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows fluorescein staining of untreated damaged eye cornea 5 days after surgery (x 16);

Fig. 2 shows fluorescein staining of untreated damaged eye cornea 12 days after surgery (x 16);

Fig. 3A shows the histological appearance of normal cornea;

Fig. 3B shows the histological appearance of untreated damaged eye cornea 12 days after surgery;

Fig. 4 shows fluorescein staining of damaged eye cornea following Intralipid™ treatment;

Fig. 5 shows the histological appearance of damaged eye cornea following Lyteers™ treatment;

Fig. 6 shows fluorescein staining of damaged eye cornea after 3 days of treatment with HDL;

Fig. 7 shows fluorescein staining of damaged eye cornea at the end of treatment with HDL;

Fig. 8 shows the the histological appearance of damaged eye cornea after HDL treatment;

Fig. 9 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lipofundin™; and

Fig. 10 shows the histological appearance of damaged eye cornea following 7 days of treatment with Lyeteers™.

persisant rubbing of the epithelium by the lids as well as severe keratitis and vascularization of the cornea.

Animal eyes that were treated with Intralipid™ show complete reversal to normal morphological structure as indicated by fluorescein staining (Fig. 4). However, animal eyes treated with Lyteer™, (Fig. 5) (commercially available artifical tears composed mainly of water and a viscous substance) did not return to normal. In contrast to normal eyes, the damaged area did not become covered again with normal layers of cuboidal epithelial cells and a single top layer of wing cells, but was covered instead by only a single layer of wing cells. These results indicate that artificial tears such as Lyteer™ cannot promote regeneration of normal eye epithelium.

In contrast to this, animal eyes treated with HDL showed essentially a complete return to normal morphological structure as indicated by fluorescein staining taken on the third day (Fig. 6) and at the end of treatment (Fig. 7) as well as by histological staining (Fig. 8). Histological staining shows essentially a complete return to normal of the eye epithelium characterized by formation of several layers of cuboidal cells and a single top layer of wing cells.

These results clearly indicate that both HDL and Intralipid™ are able to promote healing and regeneration of damaged eye epithelium and return to normal epithelium.

Example 2

Three rabbits underwent central corneal peeling of the epithelium in both eyes. The area of peeling, 8 mm in diameter, was first demarcated with trephine, and the epithelium excised with a scalpel. The right eye of each rabbit was treated with two drops of Lipofundin™ 10% three times a day, while the left eye was treated with the same dose of Lyeteers™. The cornea were stained with fluorescein and photographed immediately after epithelium removal and on the third and fifth day afterwards. On the seventh day, the rabbits were sacrificed by

a lethal dose of pental and the corneas were excised, fixed and stained for light microscopy. The denuded area in the fluorescein and fixed corneas was determined, and the extent of remaining damage calculated as the remaining denuded area divided by the initial denuded area. The results appear in Table 1 below which gives the fraction of initial damage remaining 0, 3, 5, and 7 days after peeling the epithelium. The results show that the rate of healing was faster in the Lipofundin™ treated eyes. Figs. 9 and10 show the epithelium of a Lipofundin™ treated eye and a treated eye, respectively, after 7 days of treatment. Whereas the Lipofundin™ appears close to, in the Lyeteers™ treated eye the epithelium still appears thin, immature, and containing only flat and wing cells which have migrated to the denuded area.

Table 1

	Treatment	
Time (Days)	**Lipofundin™**	**Lyeteers™**
0	1.00	1.00
3	.304	.378
5	.036	.107
7	0	0

III. Clinical study on the effect of Lipofundin™ on dry eye

Experimental

Eight female patients suffering from dry eye and presenting less than 10 mm of wet strip in a Shirmer test, were included in this study. The patients ranged in age from 62 to 74 years.

Each patient received a bottle of Lipofundin™ 5% and was instructed to instill two drops in one eye three times a day for one week, while continuing the

previous treatment in the other eye. Each patient was asked to fill out a questionnaire before and after the week of treatment in which she was to rank the following parameters on a scale of 0 (no complaint) to 4 (very severe): itching, tearing, a sensation of "sand" under the upper eyelid, and photophobia. The appearance of the anterior segments of the eyes was evaluated clinically for redness, inflammation, and discharge from the conjunctival sac.

Results

The results of the clinical studies are given in Table 2. All of the patients reported a general improvement in their symptoms and asked to continue treatment with Lipofundin™ for a longer period of time. Seven of the eight patients had no complaints about the treatment. One patient, presenting the most severe case of dry eye at the beginning of the study, complained of a burning sensation lasting about two minutes following the instillation of Lipofundin™ during the first two days of treatment. Lipofundin™ was thus well tolerated by the patients and ameliorates symptoms in people suffering form dry eye.

Table 2

Degree Symptom	Before Treatment 0	1	2	3	4	Average ± SD	After Treatment 0	1	2	3	4	Average ± SD	p value
Itching		1	3	3	1	2.50 ± 0.93	4	4				0.50 ± 0.53	0.000057
Tearing			3	2	3	3.00 ± 0.92	5	3				0.37 ± 0.51	0.000031
Sand		1	4	3		2.25 ± 0.71	6	2				0.25 ± 0.46	0.000051
Photophobia			1	4	3	3.25 ± 0.71	5	2	1			0.50 ± 0.76	0.000014

CLAIMS:

1. A pharmaceutical composition for the treatment of disorders of the anterior segment of the eye comprising, as an active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an ophthagestically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

3. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

4. A pharmaceutical composition according to Claim 3, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

5. A pharmaceutical composition according to Claim 1, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

6. A pharmaceutical composition according to Claim 5, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

7. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

8. A pharmaceutical composition according to Claim 7, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

9. A pharmaceutical composition according to Claims 1 to 8, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

10. A pharmaceutical composition according to Claims 1 to 8 wherein said agent is Lipofundin™

11. A pharmaceutical composition according to Claims 1 to 8 wherein said agent is Intralipid™

12. A pharmaceutical composition according to Claims 9-11, further comprising albumin.

13. A pharmaceutical composition according to Claim 9, wherein the HDL is human or bovine HDL.

14. A pharmaceutical composition according to Claim 9, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol.

15. A pharmaceutical composition according to Claim 9, wherein the sphingolipids are sphingomyelins.

16. A pharmaceutical composition according to Claim 9, wherein the other lipid components of HDL are triglycerides and/or glycerol.

17. A pharmaceutical composition according to Claim 9, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I,

Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

18. A pharmaceutical composition according to Claims 1 to 17, further comprising a growth factor, an attachment factor or an extracellular component.

19. A pharmaceutical composition according to Claim 18, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

20. A pharmaceutical composition according to Claim 18, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

21. A pharmaceutical composition according to Claim 18, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

22. A pharmaceutical composition according to any one of Claims 1 to 21 further comprising an agent capable of providing protection from U.V. radiation.

23. A pharmaceutical composition according to Claim 22, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

24. A method for the treatment of disorders of the anterior segment of the eye comprising administering to a subject in need of such treatment at least one agent capable of causing a net efflux of cholesterol from cells.

25. A method according to Claim 24, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

26. A method according to Claim 24, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

27. A method according to Claim 26, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

28. A method according to Claim 24, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

29. A method according to Claim 28, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

30. A method according to Claim 24, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

31. A method according to Claim 30, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

32. A method according to Claims 24 to 31, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

33. A method according to Claims 24 to 31 wherein said agent is Lipofundin™

34. A method according to Claims 24 to 31 wherein said agent is Intralipid™

35. A method according to Claims 32-34, further comprising administering albumin.

36. A method according to Claim 32, wherein the HDL is human or bovine HDL.

37. A method according to Claim 32, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

38. A method according to Claim 32, wherein the sphingolipids are sphingomyelins.

39. A method according to Claim 32, wherein the other lipid components of HDL are triglycerides and/or glycerol.

40. A method according to Claim 32, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

41. A method according to Claims 24 to 40, further comprising a growth factor, an attachment factor or an extracellular component.

42. A method according to Claim 41, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

43. A method according to Claim 41, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

44. A method according to Claim 41, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

45. A method according to any one of Claims 24 to 44 further comprising administering an agent capable of providing protection from U.V. radiation.

46. A method according to Claim 45, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

47. Use of at least one agent capable of causing a net efflux of cholesterol from cells for the preparation of a medicament for the treatment of disorders of the anterior segment of the eye.

48. A use according to Claim 47, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

49. A use according to Claim 47, wherein the disorders are manifested by physical and chemical damage to the conjunctival and corneal epithelia.

50. A use according to Claim 49, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; and conditions following damage of epithelia due to radial keratotomy.

51. A use according to Claim 47, wherein the disorders include a decrease in secretion from glands located in the conjunctiva.

52. A use according to Claim 51, wherein the disorders are selected from the group consisting of: dry eye and tear film disfunction caused by medication.

53. A use according to Claim 47, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

54. A use according to Claim 53, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

55. A use according to Claims 47 to 54, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

56. A use according to Claims 47 to 54 wherein said agent is Lipofundin™

57. A use according to Claims 47 to 54 wherein said agent is Intralipid™

58. A use according to Claim 54, wherein the HDL is human or bovine HDL.

59. A use according to Claim 54, wherein the phospholipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinosital.

60. A use according to Claim 54, wherein the sphingolipids are sphingomyelins.

61. A use according to Claim 54, wherein the other lipid components of HDL are triglycerides and/or glycerol.

62. A use according to Claim 54, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

63. A storage medium for the preservation of isolated cornea comprising at least one agent capable of causing net efflux of cholesterol from cells.

64. A storage medium according to Claim 63, wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. phospholipids and/or sphingolipids;

iii. a composition of matter comprising phospholipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iv. at least one HDL Apolipoprotein.

65. A storage medium according to Claim 63, wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. Lipofundin™

ii. Intralipid™

66. A storage medium according to Claims 64 and 65, further comprising albumin.

67. A storage medium according to Claim 64, wherein the HDL is human or bovine HDL.

68. A storage medium according to Claim 64, wherein the phospho-lipids are selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol.

69. A storage medium according to Claim 64, wherein the sphingolipids are sphingomyelin.

70. A storage medium according to Claim 64, wherein the other lipid components of HDL are triglycerides and/or glycerol.

71. A storage medium according to Claim 64, wherein the apolipoprotein is selected from the group consisting of Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

AMENDED CLAIMS

[received by the International Bureau on 2 July 1998 (02.07.98);
original claims 1-71 replaced by amended claims 1-54 (7 pages)]

1. A pharmaceutical composition for the treatment of disorders of the anterior segment of the eye, manifested by mechanical, chemical or clinical damage comprising, as an active ingredient, at least one agent capable of causing a net efflux of cholesterol from cells, together with an ophthagestically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in the conjuctiva.

3. A pharmaceutical composition according to Claim 1, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; damage to the corneal epithelium caused by a decrease in secretion of glands located in the conjuctiva; and conditions following damage of epithelia due to radial keratotomy.

4. A pharmaceutical composition according to Claim 1, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

5. A pharmaceutical composition according to Claim 4, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

6. A pharmaceutical composition according to Claims 1 to 5, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. a composition of matter termed *"reconstituted HDL"* comprising phospholipids and/or sphingolipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iii. at least one HDL Apolipoprotein.

7. A pharmaceutical composition according to Claims 1 to 5 wherein said agent is Lipofundin™

8. A pharmaceutical composition according to Claims 1 to 5 wherein said agent is Intralipid™

9. A pharmaceutical composition according to Claims 6-8, further comprising albumin.

10. A pharmaceutical composition according to Claim 6, wherein the HDL is human or bovine HDL.

11. A pharmaceutical composition according to Claim 6, wherein the other lipid components of HDL are triglycerides and/or glycerol.

12. A pharmaceutical composition according to Claim 6, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

13. A pharmaceutical composition according to Claims 1 to 12, further comprising a growth factor, an attachment factor or an extracellular component.

14. A pharmaceutical composition according to Claim 13, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

15. A pharmaceutical composition according to Claim 13, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

16. A pharmaceutical composition according to Claim 13, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

17. A pharmaceutical composition according to any one of Claims 1 to 16, further comprising an agent capable of providing protection from U.V. radiation.

18. A pharmaceutical composition according to Claim 17, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

19. A method for the treatment of disorders of the anterior segment of the eye, manifested by mechanical, chemical or clinical damage comprising administering to a subject in need of such treatment at least one agent capable of causing a net efflux of cholesterol from cells.

20. A method according to Claim 19, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in the conjuctiva.

21. A method according to Claim 19, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; damage to the corneal epithelium caused by a decrease in secretion of glands located in the conjuctiva; and conditions following damage of epithelia due to radial keratotomy.

22. A method according to Claim 19, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

23. A method according to Claim 22, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

24. A method according to Claims 19 to 23, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. a composition of matter termed *"reconstituted HDL"* comprising phospholipids and/or sphingolipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iii. at least one HDL Apolipoprotein.

25. A method according to Claims 19 to 23, wherein said agent is Lipofundin™

26. A method according to Claims 19 to 23, wherein said agent is Intralipid™

27. A method according to Claims 19 to 26, further comprising administering albumin.

28. A method according to Claim 24, wherein the HDL is human or bovine HDL.

29. A method according to Claim 24, wherein the other lipid components of HDL are triglycerides and/or glycerol.

30. A method according to Claim 24, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

31. A method according to Claims 19 to 30, further comprising a growth factor, an attachment factor or an extracellular component.

32. A method according to Claim 31, wherein the growth factor is selected from the group consisting of: Keratinocyte Growth Factor (KGF/FGF7), Epidermal Growth Factor (EGF) and other growth factors of the FGF family.

33. A method according to Claim 31, wherein the attachment factor is selected from the group consisting of: laminin and fibronectin.

34. A method according to Claim 31, wherein the extracellular matrix components are selected from the group consisting of: collagen and heparan sulfate proteoglycans.

35. A method according to any one of Claims 19 to 34 further comprising administering an agent capable of providing protection from U.V. radiation.

36. A method according to Claim 35, wherein the agent capable of providing protection from U.V. radiation is oxybenzone.

37. Use of at least one agent capable of causing a net efflux of cholesterol from cells for the preparation of a medicament for the treatment of disorders of the anterior segment of the eye, said disorders manifested by mechanical, chemical or clinical damage.

38. A use according to Claim 37, wherein the anterior segment of the eye is the corneal epithelium, stromal conjunctiva, and the glands present in both.

39. A use according to Claim 37, wherein the disorders are selected from the group consisting of: mechanical abrasion of the cornea; corneal epithelial defects created by contact lens wearing; corneal epithelial defects created by spontaneous peeling of the epithelium; corneal damage following photo-refractive keratectomy; injuries caused by chemical substances; injuries caused by ultraviolet light exposure; systemic diseases causing damage to the corneal epithelium and conjunctiva; chronic edema of cornea with recurrent erosion of epithelium; damage to the corneal epithelium caused by a decrease in secretion of glands located in the conjuctiva; and conditions following damage of epithelia due to radial keratotomy.

40. A use according to Claim 37, wherein the disorders are manifested by a slow rate of regeneration of cells of the anterior segment of the eye.

41. A use according to Claim 40, wherein the slow rate of regeneration is caused by old age, or by administration of anti-proliferative substances.

42. A use according to Claims 37 to 41, wherein said agent is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. a composition of matter termed *"reconstituted HDL"* comprising phospholipids and/or sphingolipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iii. at least one HDL Apolipoprotein.

43. A use according to Claims 37 to 41 wherein said agent is Lipofundin™

44. A use according to Claims 37 to 41 wherein said agent is Intralipid™

45. A use according to Claim 42, wherein the HDL is human or bovine HDL.

46. A use according to Claim 41, wherein the other lipid components of HDL are triglycerides and/or glycerol.

47. A use according to Claim 41, wherein the apolipoprotein is selected from the group consisting of: Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.

48. A storage medium for the preservation of isolated cornea comprising at least one agent capable of causing net efflux of cholesterol from cells.

49. A storage medium according to Claim 48. wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. high density lipoprotein (HDL);

ii. a composition of matter termed *"reconstituted HDL"* comprising phospholipids and/or sphingolipids and at least one other lipid component of HDL other than cholesterol and cholestryl-ester; and

iii. at least one HDL Apolipoprotein.

50. A storage medium according to Claim 48, wherein the agent capable of causing a net efflux of cholesterol from cells is one or more of the group consisting of:

i. Lipofundin™

ii. Intralipid™

51. A storage medium according to Claims 48 to 50, further comprising albumin.

52. A storage medium according to Claim 49, wherein the HDL is human or bovine HDL.

53. A storage medium according to Claim 49, wherein the other lipid components of HDL are triglycerides and/or glycerol.

54. A storage medium according to Claim 49, wherein the apolipoprotein is selected from the group consisting of Apolipoprotein A-I, Apolipoprotein A-IV and Apolipoprotein E or a combination of one or more apolipoproteins.